This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

1837
ARTES
SCIENTIA
VERITAS
LIBRARY OF THE
UNIVERSITY OF MICHIGAN
E PLURIBUS UNUM
TUEBOR
SI QUAERIS PENINSULAM AMOENAM
CIRCUMSPICE

LIBRARY OF THE
UNIVERSITY OF MICHIGAN

BRYN MAWR COLLEGE

MONOGRAPHS

REPRINT SERIES, Vol. I., No. 2

CONTRIBUTIONS FROM THE BIOLOGICAL LABORATORY

BRYN MAWR, PENNA., U. S. A.
December, 1902

Table of Contents.

I. On Muscle Regeneration in the Limbs of Plethedon. By Elizabeth W. Towle.
(*Reprinted from the Biological Bulletin*, *Vol. II.*, *1901.*)

II. Regeneration of Proportionate Structures in Stentor. By T. H. Morgan.
(*Reprinted from the Biological Bulletin*, *Vol. II.*, *1901.*)

III. On the Force of Contraction of the Frog's Gastrocnemius in Rigor, and on the Influence of "Chloretone" on that Process. By N. M. Stevens.
(*Reprinted from the American Journal of Physiology*, *Vol. VI.*, *1901.*)

IV. Observations and Experiments on Regeneration in Hydra Viridis. By Helen Dean King.
(*Reprinted from the Archiv für Entwickelungsmechanik der Organismen*, *Vol. XIII.*, *1901.*)

V. Growth and Regeneration in Planaria Lugubris. By T. H. Morgan.
(*Reprinted from the Archiv für Entwickelungsmechanik der Organismen*, *Vol. XIII.*, *1901.*)

VI. Regeneration in Tubularia Mesembryanthemum. By N. M. Stevens.
(*Reprinted from the Archiv für Entwickelungsmechanik der Organismen*, *Vol. XIII.*, *1901.*)

VII. Notes on Regeneration in Planaria Lugubris. By N. M. Stevens.
(*Reprinted from the Archiv für Entwickelungsmechanik der Organismen*, *Vol. XIII.*, *1901.*)

VIII. The Proportionate Development of Partial Embryos. By T. H. Morgan
(*Reprinted from the Archiv für Entwickelungsmechanik der Organismen*, *Vol. XIII.*, *1901.*)

IX. The Influence of Temperature, Odors, Light, and Contact on the Movements of the Earthworm. By Amelia C. Smith.
(*Reprinted from the American Journal of Physiology*, *Vol. VII.*, *1902.*)

147464

X Further Experiments on the Regeneration of Tubularia. By T. H. Morgan.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XIII, 1902.)

XI Experimental Studies on the Formation of the Embryo of Bufo Lentiginosus. By Helen Dean King.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XIII, 1902.)

XII The Gastrulation of the Egg of Bufo Lentiginosus. By Helen Dean King.
(Reprinted from the American Naturalist, Vol. XXXVI, 1902.)

XIII Histological Changes in Hydra Viridis during Regeneration. By Hannah Isabel Rowley.
(Reprinted from the American Naturalist, Vol. XXXVI, 1902.)

XIV The Regeneration of the Pharynx in Planaria Maculata. By Henrietta F. Thacher.
(Reprinted from the American Naturalist, Vol. XXXVI, 1902.)

XV Further Experiments on the Regeneration of the Tail of Fishes. By T. H. Morgan.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XIV, 1902.)

XVI Experimental Studies of the Internal Factors of Regeneration in the Earthworm. By T. H. Morgan.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XIV, 1902.)

XVII Regeneration in Tubularia Mesembryanthemum. II. By N. M. Stevens.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XV, 1902.)

XVIII The Relation between Normal and Abnormal Development of the Embryo of the Frog, as Determined by Injury to the Yolk-Portion of the Egg. By T. H. Morgan.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XV, 1902.)

XIX The Internal Factors in the Regeneration of the Tail of the Tadpole. By T. H. Morgan and S. E. Davis.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XV, 1902.)

XX The Follicle Sacs of the Amphibian Ovary. By Helen Dean King.
(Reprinted from the Biological Bulletin, Vol. III., 1902.)

REPRINT

FROM

BIOLOGICAL BULLETIN

VOL. II. NO. 6

BOSTON, U.S.A.
GINN & COMPANY, PUBLISHERS
The Athenæum Press
1901

ON MUSCLE REGENERATION IN THE LIMBS OF PLETHEDON.

ELIZABETH W. TOWLE.

SPALLANZANI (1768) and Bonnet (1777) showed that a salamander whose limbs have been cut off has the power to regenerate new ones. This discovery has been confirmed by later writers, and although some histological work has been done, yet the method of regeneration of the muscle bundles has not been worked out. There are several possibilities: first, the old fibers might break down at the cut ends and the new ones develop from the indifferent tissue so formed, each old muscle thus completing itself independently. Or, the cut muscles might degenerate along their entire length, and new ones take their place; or some of the old muscles might degenerate, new ones being formed from this tissue, while some fibers might break up into smaller new fibers. An attempt has been made in this work, not so much to follow the origin of the cells in detail as to discover the general processes taking place in the leg that lead to the formation of the new muscles. The regenerating limbs of *Plethedon cinereus* were used. They were studied by means of serial sections.

In addition to this histological study, I have also experimented on a number of American urodeles in order to see in which ones regeneration of the limbs takes place. For this purpose a number of the commoner forms have been studied, and in connection with these results a statement is given of the previous observations on European forms.

I.

Method. — One of the anterior limbs of *Plethedon cinereus* was removed halfway between elbow and hand. The regenerating limbs were put up at intervals varying from four days to two weeks. They were fixed in corrosive acetic, hardene for two or three days in 95% alcohol, and then decalcified f(from six to eight days in a nitric acid solution (HNO_3 sp. g 1.42, 2 vols. + H_2O, 98 vols.) which was changed daily. The were finally hardened again for three days in 95% alcohc embedded and cut. Some limbs were stained *in toto* with bor carmine, but the best results were obtained by the methc used by Byrnes ('98), *viz.*, staining on the slide in Delafield haematoxylin, followed by a wash of picric acid in absolu alcohol. This latter method differentiates the muscle substan very clearly.

Eleven stages were preserved at the following intervals:

Time of Operation	Time of Killing.	Age of Stump.
1. May 4, 1900.[1]	May 14, 1900.	7 days.
2. " " "	" 18, "	11 "
3. " " "	" 29, "	22 "
4. Oct. 22, 1899.	Nov. 20, 1899.	29 "
5. " " "	Dec. 5, "	44 "
6. " " "	" 15, "	54 "
7. " " "	" 15, "	54 "
8 " " "	Jan. 5, "	75 "
9. " " "	" 5, "	75 "
10 " " "	" 20, "	90 "
11 " " "	March 22, "	151 "

Transverse sections of this series were cut. Nos. 1, 2, ar 3 were stained with haematoxylin and picric acid; 4, 5, and with borax carmine; 7 with borax carmine and picric acic 8 and 9 with Biondi-Ehrlich solution;[2] 10 and 11 with haem toxylin and picric acid. In addition, normal limbs were c and stained in a similar manner and used for comparison.

Results. For convenience in description I shall consider th sectioned limb as made up of three Regions: I, that betwe the cut and the elbow; II, the Region just above the cut; ar III, the growing end. In the earliest stages Region III do not, of course, exist.

---

[1] [illegible] were preserved later in the same [illegible]

[2] [illegible]

In the first of the series of transverse sections changes in the cut muscles are already noticeable, the most striking being the increase in the number of nuclei, especially in the outer fibers of the limb. This increase can be seen in Region I as far up as the origin of the muscles at the elbow. In the outer fibers the muscle tissue is becoming thinner and disappearing, and while the outlines of fibers and bundles are not lost, they are much less clear than in the normal limb. The inner bundles, however, are but little affected, and extend unbroken to the cut end, which is at this time not yet entirely covered by ectoderm. No mitosis is seen in this section.

In stage 2 the changes are more marked. There has been a continued increase in the number of nuclei in Regions I and II, the outlines of the outer fibers and bundles are lost, while the muscle substance has disappeared except for disintegrating fragments here and there, contrasting sharply with the thin cytoplasm of the neighboring cells. The inner fibers still extend to the end of the limb, which is now covered entirely by several layers of ectoderm. In the neighborhood of the cut three or four mitotic figures are to be found. In the third stage Region III begins to appear as a small knob of undifferentiated tissue behind the cap of ectoderm. In this knob and for a short distance above it among the outer cells karyokinesis is not uncommon.

If we compare the following stage (4) with the normal limb, the principal changes that have taken place will be very clearly brought out. In Region I the increase in number of nuclei is very great. Even as far as the elbow two to four nuclei may be found in a section of a single fiber, often crowded together so as almost to fill it (Fig. 1). Many nuclei are also scattered between the fibers. Below the elbow the number of nuclei increases, the outlines of the outer fibers are completely lost, and the outer half of the limb, which is normally solid muscle, is seen to be made up of a dense mass of nuclei surrounded by loose protoplasmic substance, with here and there

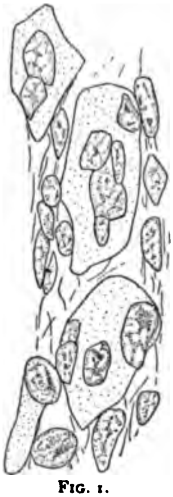


Fig. 1.

clumps of disintegrating muscle tissue. The outlines of the inner fibers are somewhat less distinct than in the preceding stage and some of the bundles seem to be splitting up. This condition is represented in Fig. 2. Most significant is the fact that at no stage is any karyokinesis found among the muscle fibers although the increase in the number of nuclei is enormous. Passing through Region II of this stage, the number of old fibers decreases and the scattered nuclei increase, until at the plane where the cut was made all old fibers disappear and we reach Region III, which is made up entirely of closely crowded nuclei, each surrounded by a small amount of protoplasm. Karyokinesis is first seen in II, a short distance above the cut, among the outer cells, *never in the muscle fibers*, and the number of dividing cells increases toward the growing tip until it becomes quite large.

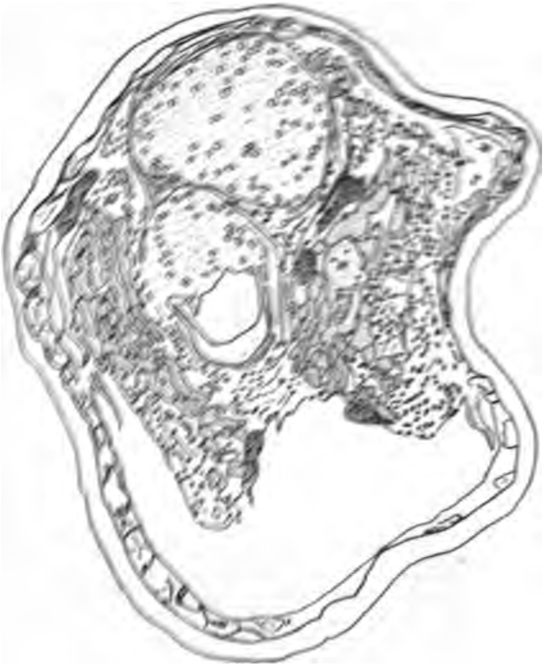


FIG. 2.

In the fifth stage a further difference is to be noted. We find in Region I, in the inner part of the limb, the old muscle fibers, often with several nuclei, and broken frequently into quite small fragments. Outside these are numerous nuclei, as before, but now surrounded by very distinct muscle tissue (Fig. 3). This tissue has formed often about several nuclei in a group, and has no distinct walls; there seem to be as yet no distinct muscle fibers. But the line of separation between the old muscle fibers and the new tissue is distinct, and a few old fibers can be traced to the region of the cut, although at that level the greatest part of the tissue is new. New muscle substance has appeared about the nuclei for a short distance below the cut; but it decreases as we pass down, until we find only crowded nuclei and thin protoplasm. In this region, as before, numerous karyokineses are seen.

In Region I of the sixth stage all the muscle fibers are small and the definite line between the old and new is lost. The majority of the fibers contain in cross-section but one nucleus, though some may contain two or three, and in general the smallest fibers and most nuclei are on the outer side of the limb. This is especially noticeable in Region II, where the outer (new) fibers are exceedingly small. The muscle tissue decreases in amount as we pass to Region III, until it is all lost. Cells dividing by karyokinesis appear at this level.

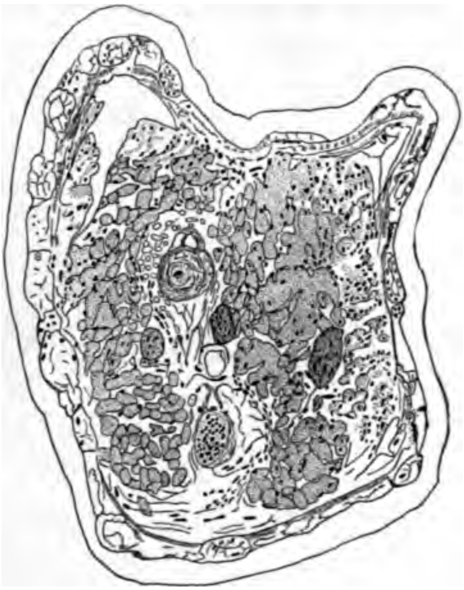


FIG. 3.

Stage 7, though of the same age as the preceding, is somewhat further differentiated, and in this the new fibers are more rounded and have assumed a more characteristic form (Fig. 4). A comparison with the normal shows smaller fibers and great amount of nuclei, there being often two or three in one fiber and many outside the fibers.

The next stages need not be described in detail. As the limb grows larger the formation of new muscle tissue progresses farther down toward the tip, the new fibers being always small and containing several nuclei. The number of nuclei outside the fibers decreases, until in stage 11 the muscles are quite normal, and the number of nuclei is excessive only in the region of the foot, which is at this time

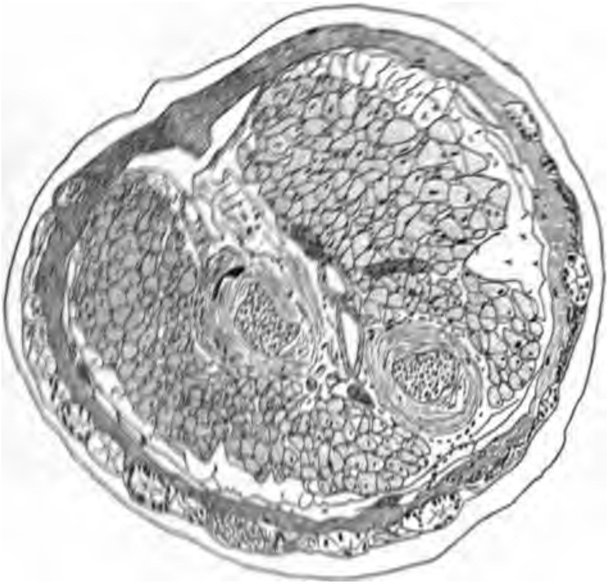


Fig. 4

clearly differentiated. Karyokinesis is found, I believe, without exception, near the growing end, never in the upper regions.

The first appearance of any definite grouping of cells appears in stage 5, where the arrangement into bundles is foreshadowed. As the fibers form, the division into bundles becomes more distinct, until in stage 11 they are all differentiated as far down as the foot, and here we can see by the arrangement of nuclei where the bundles are to be.

In the process of regeneration described above there are certain points to which I wish to direct especial attention. In the first place there is a great increase in the number of nuclei *within the old fibers,* but in no case is any karyokinesis found there. This degenerative process in the old fibers must therefore take place by direct division of the nuclei. Instances of this division are shown in Figs. 5 and 6. To this division and to the disintegration of some of the old fibers is due the enormous accumulation of nuclei in the outer part of the limb (Fig. 2). The cells so formed then begin to divide by karyokinesis in the region of the cut, and thus a further increase in their number takes place. In these outer cells new muscle tissue forms and the new fibers are built up. A certain number of the old fibers remain in the middle of the limb, and in these the muscle tissue never disintegrates, though it splits longitudinally.

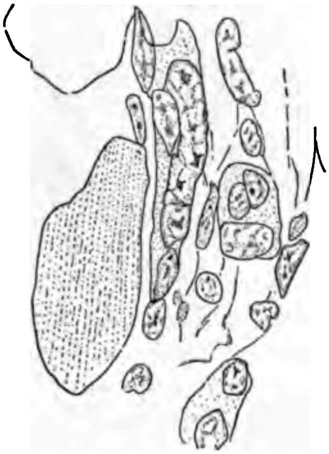


FIG. 5.

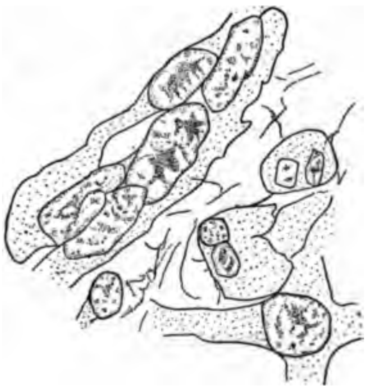


FIG. 6.

Again, as at any one level the number of nuclei far exceeds the number of fibers in a normal muscle, a great number of them must, between the early stages and the fully formed limb, either degenerate or be transported (*cf.* Figs. 3 and 4). That this is so is easily seen, for two reasons: (1) when the new fibers form, at a given level several nuclei are often included in one fiber ; when the limb is full-grown there is only one ; (2) among the newly formed fibers, but *between* them, are many scattered nuclei ; the majority of these disappear in later stages.

One further point should be mentioned. In stage 5 the line of distinction between new and old fibers is clear, owing to their difference in size. In stage 7 this distinction has disappeared. This is due not only to an increase in the size of the new fibers, but to a decrease in the old. This decrease is, I believe, due to the longitudinal splitting of such of the old fibers as are left (Fig. 7. *a* and *b*).

Beside the stages described above, four others were preserved and cut longitudinally. This was a somewhat difficult operation, for the new part forms at an angle with the upper arm, and it is hard to orient the piece in such a way as to insure true longitudinal cutting of the muscle fibers. The material was preserved at the following intervals:

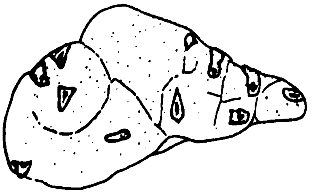


FIG. 7 *a*.

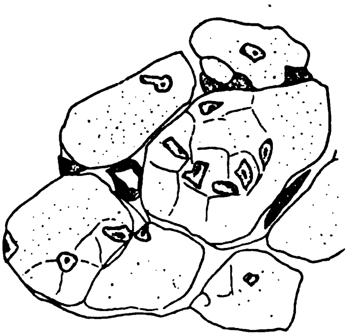


FIG. 7 *b*.

Time of Operation.	Time of Killing.	Age of Stump.
1. Jan. 29, 1900.	Feb. 2, 1900.	4 days.
2. " " "	" 12, "	14 "
3. " " "	" 19, "	21 "
4. " " "	" 28, "	30 "

The first of these shows no transformation at the cut ends of the muscles, but the ectoderm has closed in over the wound. In the second there is a considerable increase in the thickness of the ectoderm, and under it a collection of scattered nuclei, exactly similar to the tissue in the growing end of later stages. As the limb grows in length this tissue increases in amount and a number of mitoses are seen in it. Degeneration of the old fibers is distinctly noticeable in stage 3, and fibers are found, as before, filled with nuclei, but there is no karyokinesis in any muscle fiber.

SUMMARY.

The main changes that take place in the muscles of *Plethedon cinereus* during the process of regeneration are as follows :

1 (*a*) In the cut muscles the nuclei divide directly and in the outer bundles the fibers disintegrate, leaving masses of nuclei with a small amount of cytoplasm. (*b*) Some of the cells so formed later divide mitotically, and by them new muscle substance is laid down. (*c*.) As the number of nuclei is, however, far in excess of the normal number of muscle fibers, many nuclei must degenerate or be transported.

2. The fibers of the inner bundles do not disintegrate, but split longitudinally, giving rise to smaller fibers, which are soon indistinguishable from those formed as described in 1.

3. The arrangement into muscle bundles first becomes clear at the end of about six weeks.

4. There is no change in the muscles of the upper arm.

II.

The following is a brief summary of the main observations that have been made on the power of regeneration of the limbs in European species of salamanders.

Spallanzani (8), in 1768, published a number of observations on aquatic salamanders, presumably species of Triton. In these he found that any or all of the limbs will regenerate, no matter what the species, size, or age of the animal, the larger ones regenerating more slowly than the smaller forms. Regeneration will take place, he maintained, even when the limbs are disarticulated.

Bonnet, 1777 (2), confirmed Spallanzani's observations, finding that in *Triton cristatus* the hands and fingers will regenerate.

Von Siebold (7), in 1828, recorded abnormal regeneration of the fingers of *Triton cristatus.*

Higginbottom, in 1847 (4, p. 29), observed that in Triton the limbs will regenerate at a temperature of from 48° to 57° F.

Philippeaux (6), in 1866, was the first to prove conclusively that limbs when disarticulated will not regenerate. His experiments were made on *Triton cristatus* and Axolotl.

Dumeril (3), in his paper of 1867, in the course of other observations, notes the fact that in an Axolotl the two anterior limbs regenerated after injury.

Wiedersheim, 1875 (1, p. 95), found that the toes of *Triton cristatus* will regenerate,.while there is no regeneration of the limbs in Proteus and *Siren lacertina.*

Erber, 1876 (4, p. 34), notes regeneration of the feet of *Siren lacertina.*

Goette, 1879 (5), records the regeneration of a leg of Proteus after a year and a half. Regeneration also occurs in Amphiuma and Siren, in *Triton cristatus*, *T. taeniatus*, and their larvae.

Weismann (9), in The Germ Plasm, 1893, says that the limbs of *Salamandra* regenerate, while in *Triton marmoratus* regeneration is slight or absent.

Barfurth (1), in 1894, reports regeneration of the feet and digits of *Triton taeniatus* and *Siredon pisciformis.* This regeneration is normal or abnormal according to the plane and method of the injury.

I have experimented on the following forms, removing a fore foot and a hind foot from different individuals of each species: *Plethedon cinereus*, *Spelerpes ruber*, *S. guttolineatus*, *Desmognathus ochrophaea*, *Manculus quadridigitatus*, *Amblystoma opacum*, *Diemyctylus viridescens*, *Amphiuma means*, and *Necturus maculatus.* Of these, all have regenerated.[1] The regeneration in Spelerpes, Desmognathus, Manculus, and Amblystoma was comparatively rapid, and new limbs were well formed in four months, though they were somewhat smaller than the old limbs. Diemyctylus was slower in reaction, while the first Necturus to show a distinct regenerated stump did so only after eight months. Other individuals of the same species showed no regeneration even at that time.

I desire to thank Professor T. H. Morgan, under whose direction this work was undertaken, for his kindly assistance during its progress.

BIOLOGICAL LABORATORY OF BRYN MAWR COLLEGE.

---

[1] *Amphiuma means* was observed for only eleven weeks. At that time regeneration was slight. The regenerated stump was not sectioned.

LITERATURE.

1. **Barfurth.** Arch. f. Entwickelungsmechanik der Organismen. 1895. p. 91.
2. **Bonnet.** Mémoire sur la reproduction des membres de la salamandre aquatique. *Œuvres completes.* Tome xi. I. Mémoire. 1777.
3. **Dumeril.** Nouvelles archives du muséum d'histoire naturelle de Paris. Tome iii. 1867.. p. 119.
4. **Fraisse.** Die Regeneration von Geweben und Organen bei den Wirbelthieren. Cassel und Berlin. 1885.
5. **Goette.** Ueber Entwickelung und Regeneration des Gliedmassenskeletts der Molche. Leipzig. 1879.
6. **Philippeaux.** Comp. Rend. 1866. p. 576.
7. **Siebold, Von.** Observationes quaedam de salamandris et tritonibus. *Dissert. Berolini.* 1828.
8. **Spallanzani.** Précis sur les reproductions animales. 1768. p. 79.
9. **Weismann.** The Germ Plasm. New York, Charles Scribner. 1893.

REGENERATION OF PROPORTIONATE STRUCTURES IN STENTOR.

T. H. MORGAN.

THE important results of Gruber and of Balbiani on the power of regeneration of pieces of *Stentor coeruleus* opened the way for further experiments; and the works of Johnson and of Lillie on the same form have added some further results of interest. There remained, however, one problem that had not been touched upon by these investigators, an answer to which is needed to make more complete our knowledge of the regeneration of unicellular forms. I refer to the question of the proportionate development of the new organs in pieces of different sizes, and from different parts of the body; and also the no less important question of the change in size of old organs that may be present on the piece at the time of its removal. It is the purpose of the present communication to describe certain experiments that bear on these questions.

Although it is evident, in a general way, from the figures given by Gruber and by Balbiani that a small piece produces a smaller peristome than does a large piece, yet their figures do not show definitely that such is the case, and, in fact, it would be difficult to determine that such is the case from observations made on the swimming animals. The figures that have so far been published represent the new stentor as it appears while contracted or when swimming. To obtain sufficiently accurate data for the problems that I wished to examine, it was necessary to make the measurements and drawings from the stentor at rest when in a fully expanded condition. The object of my work was to find an answer to the following questions: 1. Do small pieces produce a new organism having the typical proportions of the normal; and does it make any difference in this respect as to the part of the stentor from which the piece is taken? 2. If a piece containing the old peristome is cut off, will it retain the old peristome, or absorb it and produce a new one of proportionate size? If the old peristome persists, will it decrease in size until it has assumed the typical proportions? 3. If a part only of the old peristome is left on a piece, will the missing parts be regenerated from it, or will a new peristome develop?

There appeared during January and February in one of the aquaria in the laboratory a large number of stentors, whose presence seemed to be connected with the appearance of vast numbers of vorticellas, on which they fed. The operation of cutting the stentor in two or more parts was carried out either by means of small scissors, or, in most cases, by a sharp scalpel. The latter operation is greatly facilitated by placing the animals in a dish of water, the bottom of which is covered by a layer of paraffin.

The following measurements give the length of the normal blue stentor and the greatest width of the peristome.

Length.	Width of Peristome.
2.8 mm.	.52 mm.
1.6	.46
1.4	.40
1.7	.50
1.7	.48
1.6	.44
1.9	.48

If a stentor is cut in two by a cross-cut, as indicated in Fig. 1, *A*, *a–a* (the anterior piece, *B*, being smaller than the posterior, *C*), the cut surfaces of each piece are closed almost instantly by the outer layer bending over the exposed part. Only a faint, clear line on the surface indicates where the cut has been made. The history of the anterior piece is as follows: In the course of an hour or two the piece becomes somewhat more pointed at the posterior end, and then fixes itself by a foot that appears at that end (Fig. 1, *B*). The posterior end now begins to draw out into a stalk, and after thirteen hours the piece has assumed the form shown in Fig. 1, *B*¹. The piece is still proportionately too broad for its length, for although the peristome has become reduced in size it is not as

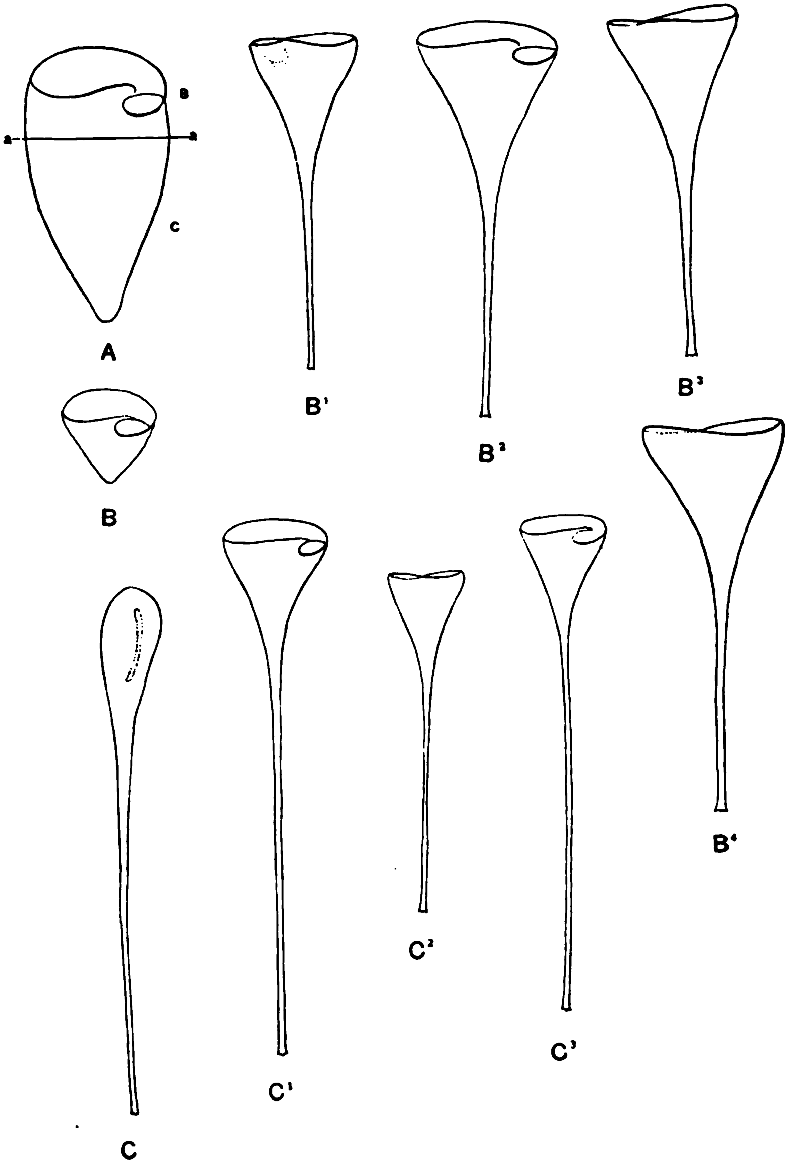


FIG. 1.—*A*, contracted stentor to show where the cut was made, *a-a*; *B*, anterior end five hours after the operation; B^1, an anterior piece thirteen hours after operation; B^2, B^3, anterior pieces twenty-four hours after operation; B^4, anterior piece forty-eight hours after the operation.

C, posterior or foot end of *A*; C^1, posterior end five hours after operation; C^2, C^3, posterior ends twenty-four hours after operation.

yet reduced sufficiently to give the piece the typical proportions. Two other head-pieces of this same series are shown in Fig. 1, *B*^{2}, *B*^{3}, that were drawn twenty-four hours after the operation. It is even more evident in these (compare with Fig. 2, *A*, for normal) than in the last that the peristome is too broad for the length of the stentor. Even after another twenty-four hours one of the pieces had still retained the same form as shown in Fig. 1, *B*^{4}.

The posterior piece, *C*, fixes itself at once by the old foot, and may soon elongate to its full length. In the course of two or three hours a clear band appears extending somewhat obliquely over the rounded end of the piece (Fig. 1, *C*). Cilia appear along the band. In a few more hours, the rate depending on the temperature, the ciliated band moves forward around the anterior end of the piece, and in doing so bends around on itself into the characteristic peristome. A new peristome-field, or disk marked by delicate parallel lines, appears on the inner side of the band even before it moves forward, and as the band bends around to make the terminal peristome, the new disk comes to lie in its central part. A depression, that appears at the basal end of the band, forms the pharyngeal funnel. The new peristome is smaller than that of the original animal, and, as the figures show (Fig. 1, *C*^{1}, *C*^{2}, *C*^{3}), it is, in some cases, even smaller than the reduced peristome on the anterior piece. The foot-piece is also at first very long as compared with the size of the new peristome ; and this condition may remain for several days.

These results demonstrate that, for some time after the new organs have developed, the new stentors retain some of the peculiarities of the part of body from which they have come. When the pieces are contracted, or are swimming, these relations are scarcely evident and might easily escape detection.

The transformation of an anterior piece into a new stentor is much more strikingly seen when only a small part of the anterior end is cut off. One set of observations on the same individual is represented in Fig. 2. The stentor fully extended is represented in Fig. 2, *A*. The anterior end had been cut off, as shown in Fig. 2, *A*^{1}, while the animal was contracted.

After its removal the anterior end, *B*, contracted still more, so that its posterior cut-surface was quickly covered over by the bending in of the sides; the disk bulged forward. After a few hours the piece became somewhat pointed at its posterior end, and then fixed itself by a foot that appeared at the end.

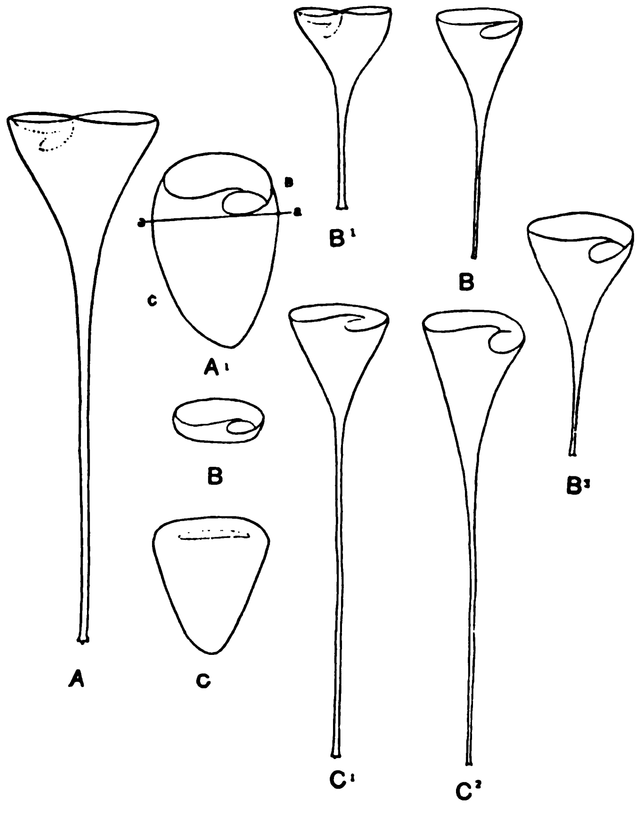


Fig. 2.—*A*, 1.4 x .4 mm. Stentor fully extended; *A*¹, same contracted, cut in two at *a*–*a*; *B*, *C*, immediately after operation; *B*¹, .56 x .26, anterior end after twenty-four hours; *B*², .7 x .25, same after another forty-eight hours; *B*³, .64 x .28, same after another four days, *i.e.*, seven days after operation; *C*, 1.2 x .27, posterior end after twenty-four hours; *C*², 1.2 x .20, same after another forty-eight hours.

After twenty-four hours (it had been kept in the cold overnight, *i.e.*, for ten hours) it appeared as shown in Fig. 2, *B*¹. The old stentor measured 1.4 mm. by .4 mm. This new stentor measures .56 mm. by .26. The old peristome has, therefore, decreased nearly to half its original width. Two days later (Fig. 2, B^2) the stalk was somewhat longer, and after another four days (Fig. 2, B^3) the form had not materially changed. The development of the posterior piece of this same individual is shown in Fig. 2, C^1 and C^2. The piece is about twice as long as the anterior piece, but its peristome is about the same size.

A similar operation was carried out on another individual; the results are shown in Fig. 3, *A*. A very small part of the anterior end was cut off (Fig. 3, A^1). It contained, however, the entire peristome and disk (Fig. 3, *B*). About thirty hours after the operation the anterior piece appeared as shown in

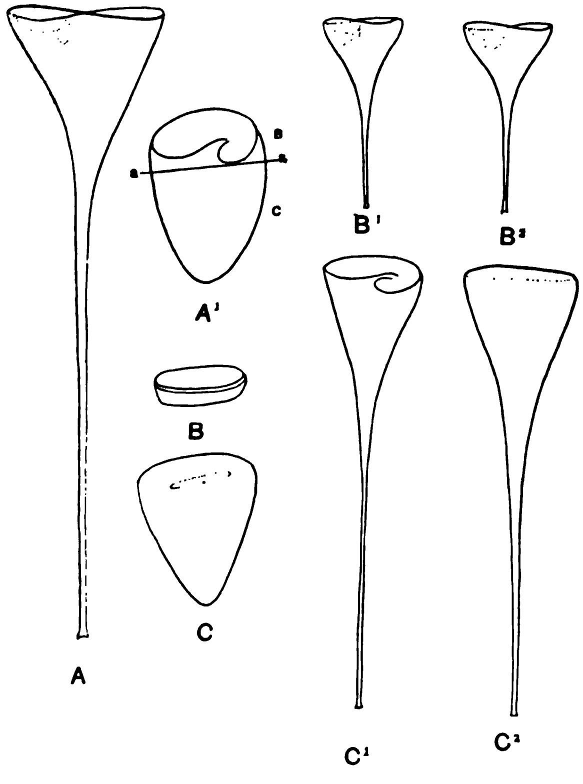


FIG. 3.—*A*, 1.7 × 4. Stentor fully extended; A^1, same contracted, cut in two at *a–a*; *B*, *C*, immediately after operation; B^1, .5 × .21, five hours after operation; B^2, .5 × .24, three days after operation; C^1, posterior piece, 1.2 × .27, five hours after operation; C^2, same, 1.6 × .32, three days after operation. In Figs. 2 and 3 one individual used in each. In Fig. 1 several individuals were used.

Fig. 3, *B*[1]. After three days more the new stentor had about the same form. The peristome is, as compared to that of the original stentor, too wide for the length of the new individual, although it is not much more than half the width of the old peristome.

The development of the posterior piece is shown in Fig. 3, C^1 and C^2. In this piece, particularly in the earlier stage, C^1, the peristome is smaller than was the original peristome, and also relatively smaller as compared with the entire length of the animal.

In order to be certain that the anterior pieces did not produce new peristomes during the night, they were kept in a cold place when not under observation; for I had found that under these circumstances the formation of a new peristome is greatly delayed, even although it may have begun to develop before the piece is subjected to the cold. In this way I could retard the development of the peristome for twelve hours, so that I felt certain that a new peristome had not developed on these anterior pieces in my absence.

In other experiments pieces of different sizes were cut from the foot-end in order to see if the size of the new peristome that is formed is in proportion to the size of the piece. It was found that a smaller peristome develops on a smaller piece, and a larger one on a larger piece; and this same relation holds also for pieces of different sizes for other parts of the body. It has been shown that cross-pieces from the anterior or posterior ends retain some of their original peculiarities even after the formation of a new individual, and that for several days the stentors from anterior pieces are too broad for their length, and individuals from the posterior end are too long for their breadth. Some of these newly regenerated stentors from the anterior pieces were kept for a longer period and supplied with food. Their measurements for from one to seventeen days after the operation are given in the following table. The measurements of three normal individuals of this lot were 1.6 × .5; 1.4 × .4; 1.1 × .4. The experiment began February 3.

ANTERIOR [illegible]

[illegible]

It will be seen from this table that, after feeding, the stentors from anterior pieces grew larger. The increase takes place both in the peristome and in the length of the piece, so that the proportionate size of the disk to the rest of the piece remains about the same. The new stentors had begun to divide on February [illegible], and by February [illegible] there were about twice as many present as at first. They have, however, about the same proportionate size as at first. The question arises whether in the normal stentor the ratio of the breadth of the disk to the length of the body may not be less than in very large individuals. [illegible] measured some of the smaller individuals found in the aquaria with the larger ones and obtained the following results: [illegible] 28 [illegible] 72 × 3[illegible] [illegible] 28. There is [illegible] some variation in the relative size of the length to the breadth [illegible] in part to the individuals not always [illegible] the same extent, and also in part to some of the measurements [illegible] not having been made in [illegible] differences, as some [illegible] measurements have shown. It will be seen that [illegible] the greatest breadth of the peristome [illegible] nearly [illegible] of the total length, in the [illegible] breadth is more nearly one-third of the [illegible] the peristome is proportionally somewhat [illegible]. Comparing these measurements [illegible] from pieces of [illegible] that they have reached in several cases [illegible] *for a small individual*. Since there is a good deal of variation in the proportion between the width of the peristome and the length of the animal both for small normal individuals and for those that have come from anterior pieces, it may be stated that pieces from the anterior end may produce new stentors whose proportions come within the range of variation of size of normal small stentors of about the same length. The measurements of posterior pieces, that are at first too long for the size of the peristome, show that they, too, assume more typical proportions. Thus one of the posterior ends of the last series measured, on February 6, 1.0 × .25. On February 10, two other individuals in the same list measured 1.4 × .38 ; 1.2 × .4. The peristomial region had, therefore, reached the full size.

A somewhat crude comparison may bring the results home. If a man were cut in two at the waist and the pieces behaved in the same way as those of stentor, two new individuals would develop. The anterior half would produce a small man with a head too large for his height, *i.e.*, his legs would be too short for a man with that sized head. Although the old head had grown smaller, it would be still too large for the rest of the new man. In fact, his proportions would be more like those of a baby whose head is relatively too large for his length as compared with that of a man, and his legs too short. It is just this result that we have found for anterior pieces of stentor. If the new man were supplied with food, all parts of the body would grow larger; but as he got larger his legs would grow faster than his head.

The posterior end of our imaginary man would have at first legs too long for his total length, and his new head would be relatively too small; but if he were fed his head, shoulders, and arms would grow faster than any other part and continue to grow until the proportionate size had been reached. If he were not fed, it is possible that his head and upper part might increase more slowly in size at the expense of the material in his legs, and the latter would get smaller until a balance was reached. The result would be that a boy rather than a baby was produced.

In other experiments pieces were removed that contained only a part of the peristome. In one series these pieces were cut off, as shown by the line *a–a* in Fig. 4, *A*; so that there was a smaller and a larger piece, the former, *B*, containing a part of the old peristome, but not any part of the pharyngeal funnel, and the latter, the larger piece, *C*, containing also a part of the peristome as well as the funnel. The cut surface of each piece is quickly closed by the bending in of the sides, and the cut ends of the peristome are generally brought together to make a closed ring. A foot develops on the basal end of the anterior piece, *B*, B^1, and a stalk is soon produced in that region. This piece may remain for twenty-four hours in this

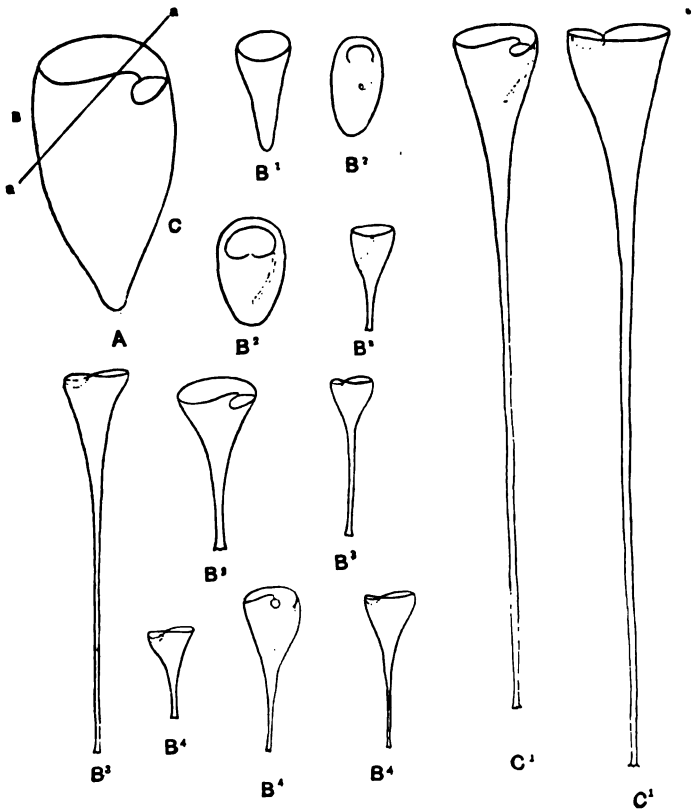


FIG. 4. — *A*, stentor partially contracted, cut in two at *a–a* into small piece, *B*, and larger, *C*; B^1, piece *B* after seven hours; B^2, B^2, B^2, piece like *B* after twenty-four hours; B^3, B^3, B^3, same after twenty-nine hours; B^4, B^4, B^4, same in part after forty-eight hours (after operation). C^1, C^1, piece *C* after seven hours.

condition. Sooner or later a new ciliated band appears on the old wall behind the part of the old peristome, as shown in Fig. 4, B^2. The band moves forward, fusing with the old ring at one point and, replacing the latter, produces a new peristome. Whether the piece of the old ring is entirely obliterated, or whether a part of it remains to contribute to the new peristome, I did not determine. The regeneration of a new peristome on these pieces may be delayed for several days (Fig. 4, B^4), and, in general, does not appear as soon as on pieces that do not contain any part of the old peristome. Five or six series of experiments of this sort, each series of a number of pieces, were made, and the smaller pieces followed with great care. Only those smaller pieces were isolated that contained no part of the old funnel. Nearly all the pieces behaved in the way just described, but in one or two a small funnel developed where the cut edges came together. This may have been due to a very small piece of the original funnel having been cut off, or to the piece having come from very near to the old funnel, or, as seems more probable, to the development of a new funnel from the old ciliated band. If the last interpretation is correct, it shows that in exceptional cases the peristome may complete itself. In the large majority of cases this does not occur and a new peristome and funnel develop at the side and move forward. In nearly all cases the cut ends of the old peristome come together, meeting in a slight notch. In one or two instances one band lay slightly below the other at the meeting point, producing a peristome exactly like the normal in shape, only the funnel was absent. If a small piece of the old funnel is left, it assumes the characteristic position of the funnel, and, in fact, becomes such to all appearance, although this peristome is generally replaced later by a new one.

Gruber studied the regeneration of pieces somewhat similar to these without a funnel, and states that the remaining part of the old peristome gives rise to a new one, but I have not found this to be the case. If the piece is kept under close observation, the development of a new peristome is found to take place in the way just described. The change is sometimes so rapid that a few hours may suffice to bring it about.

The history of the complementary piece (Fig. 4, *C*) is as follows: After the cut surface has been closed over and the edges of the peristome brought together, the piece may immediately fix itself by the old foot. The piece elongates to its full length, which is the same as that of the former animal (Fig. 4, C^1). In some of these pieces I have observed the development of a new peristome in the course of a few hours after the operation (Fig. 4, C^1). It seems that this takes place sooner when only a small part of the old peristome and funnel is left than when a larger part remains. In cases in which a large part of the old peristome remains a new peristome may not develop for several days; and in some cases I have not found it to appear at all, but I cannot state positively that it does not ultimately appear. Since even normal individuals may produce a new peristome, the appearance of a peristome on these new stentors after several days may be only the regular process of renewal of that organ. In two cases, in which a new peristome appeared after two days, the old one had begun to break down while the new band was developing. In all other cases the old part was still active and normal in appearance up to the time of its replacement by the new ciliated band. These results show that even in a large piece the new peristome is not regenerated from the old one. The presence of the old pharyngeal funnel in these pieces does not make any important difference in the end result, although it may be that pieces of this sort regenerate less quickly than when the piece does not contain the funnel portion of the old peristome.

Another experiment that supplements the preceding one in several respects consists in cutting the stentor in two, as indicated by the line *a–a* in Fig. 5, *A*. In this case the smaller piece, *B*, contains the funnel part of the peristome, while the larger piece, *C*, contains the remaining part of the peristome.

The smaller piece, *B*, closes in, develops a foot, becomes attached and produces a stalk. The edges of the peristomial ring unite more or less, as shown in Fig. 5, B^1. In one case a new peristomial band appeared six hours after the operation, moved forward, and produced a new peristome (Fig. 5, B^1). In other cases, in which the piece was small in comparison to the size of the remaining part of the peristome, a new peristome did not appear in one case until after thirty hours (Fig. 5, B^4); in other cases a new peristome had not appeared at this time (Fig. 5, B^3).

The complementary piece (*C*, Fig. 5) closed in, fixed itself, and extended to its full length. In pieces in which the remaining part of the peristome, that had united to make a ring, was quite small a new ciliated band appeared in four hours; in others, in six hours (Fig. 5, C^1 C^1); and in pieces with a larger peristomial region, after twenty-four and even after fifty-one hours (Fig. 5, C^3). It is interesting to note that in these pieces the region from which in the normal individual the peristomial band is formed has been more or less completely removed, yet a new peristomial band may very quickly

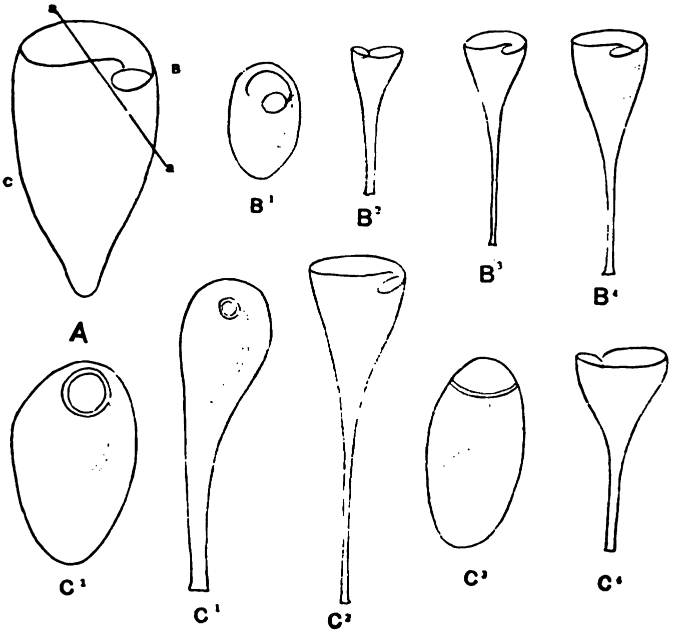


FIG. 5.—*A*, stentor partially contracted, cut in two at *a–a* into a small piece, *B*, and a large piece, *C*; B^1, piece like *B* after seven hours; B^2, piece like *B* after twenty-seven hours; B^3, B^4, piece like *B* after twenty-nine hours; C^1, C^1, piece like *C* after seven hours; C^2, piece like *C* after twenty-nine hours; C^3, C^4, piece like *C* after fifty-one hours.

appear. I did not attempt to determine the position of the new band in its relation to the region of closure of the piece.

In several cases in the last two experiments, and in some other experiments like those shown in Fig. 6, *A*, B^1, C^1, small pieces were sometimes cut off that contained a part of the old peristome, but which did not fix themselves, or assume the characteristic form. As these pieces were generally small, although not below the minimal size, there can be little doubt that most of them did not contain any part of the nucleus, and in several cases I proved this to be the case by staining the pieces in picro-carmine. The result shows that in the absence of the nucleus a piece containing a part of the old peristome cannot complete the peristome from the remaining part. This is the less to be expected since it has been shown that even in nucleated pieces the new peristome is produced not by the old one, but by the development of a new peristomial band. The result is interesting in connection with a result obtained by Gruber, *viz.*, that if a non-nucleated piece containing a part of the newly forming ciliated band is obtained it produces from the band a new peristome. My results show that a piece of the old band cannot act in this way. That the presence of the nucleus is connected with the formation of a new peristomial band seems highly probable, but I can easily imagine that could a non-nucleated piece be supplied with certain unformed elements it might be capable of producing a new peristome. The results do not seem to me to show more than that the nucleus supplies certain products of metabolism that must be present before the protoplasm can successfully carry out its

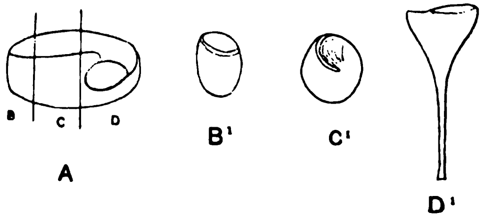


Fig. 6. — *A*, anterior end of stentor cut off and then divided into three pieces. Two of these, B^1, C^1, were apparently without nuclei, and did not produce a new peristome or assume the typical form.

innate tendency to complete the typical form. We are not justified, I believe, in drawing the conclusion, as Gruber has done, that preformed elements of the peristome exist in the nucleus and must be set free in order to initiate the development of a new peristome.

Lillie has found that the smallest piece of *Stentor polymorphus* that becomes a perfect form is equal to a sphere of about 80 μ in diameter. The average size of the stentors was equal to 230 μ. This makes the volume of the smallest stentor about $\frac{1}{27}$ of the normal. For *Stentor coeruleus* the smallest stentors measured 90 μ (= $\frac{1}{11}$ mm.), the average normal stentor 280 μ (= $\frac{7}{25}$ mm.). Therefore the former is about $\frac{1}{27}$ of the latter.

Although I have not worked specially on this problem, yet I have obtained some small stentors that were proportionately smaller than those obtained by Lillie. Thus one individual measured when extended .25 × — .08 mm., and when contracted into an oval or nearly into a sphere .08 × .08 (= $\frac{1}{12}$ mm.). The larger normal stentors measured about .4 × .32 × .32 when contracted. Although it is only possible to give a general estimate of the relative size of these two individuals, the smaller cannot be over $\frac{1}{64}$ of the former. It would be a mistake to infer from this, as well as from Lillie's calculations, that the latter came from a piece $\frac{1}{64}$ or even $\frac{1}{27}$ of the original stentor. The protoplasm of stentor is so vacuolated that a piece losing the fluid in the protoplasm might become much smaller than when first removed.

Lillie states that he believes that it would be possible to obtain a smaller individual of *S. coeruleus* than $\frac{1}{11}$ mm. The one that I obtained was in fact somewhat smaller, *viz.*, $\frac{1}{12}$ mm. The difference in our results depends, therefore, rather on the size of the normal average stentor with which the comparison is made than on the smallest individual obtained. Lillie says that he does not think there can be much difference in the absolute size of the smallest stentors, whether one uses the largest or the smallest normal specimens. It seems to me that this may or may not be true, according to what factors may enter into the result.

The conclusion that a piece $\frac{1}{27}$ or even $\frac{1}{64}$ of the entire animal can produce a new individual can give only a most general idea of the relative size of the smallest piece, since more depends on the size of the normal individual than on that of the smallest pieces, and there is for stentor a very wide range of size that may be called normal. A large normal individual may contain eight times the volume of a small normal individual. More significant, therefore, is the absolute size of the smallest piece capable of regeneration, and in this respect my results are practically in accord with those of Lillie.

Several experiments were made in which pieces were cut in two longitudinally. In a longer or shorter time most of the halves produced a new peristomial band that became a new peristome. As this experiment did not give promise of much that is new, it was tried in only a few cases.

A few casual observations made during the course of the work may be briefly mentioned. The stentors were observed dividing on several occasions, but Johnson's excellent figures and account of these stages leave nothing new for me to add. I have often noticed that after division the two products are found attached side by side, and if they are not disturbed a little colony may arise in the same spot. Several times I have observed that two individuals that have been formed by division of one of the regenerated posterior pieces were unequal in size, although I do not know whether the smaller individual was the distal or the proximal one.

As Gruber has pointed out, the first steps in the process of division and of regeneration are the same, and this holds also for the physiological replacement of the old peristome. In all cases a peristomial ciliated band appears *on the side* and moves forward around the anterior end to become the new peristome. We have here another illustration that shows that during the process of regeneration the factors that appear in the normal growth may take part in the regeneration, and this relation appears to hold for unicellular as well as for multicellular forms.

In many cases, especially where a somewhat oblique cut has been made, the superficial blue stripes come together over the cut surface in a most irregular way, yet this does not appear to interfere with the subsequent regeneration ; and after a time the stripes appear to be more regularly arranged. That a certain amount of absorption takes place, and possibly also development of new stripes, seems probable, but I have not studied these changes in any detail. It would be interesting to find out if in cross-pieces of the body the number of the stripes remains the same and their size becomes smaller, or whether the number of the stripes is proportionately reduced.

On several occasions I have tried to graft together pieces of different stentors, but the exposed surfaces close so quickly that I have not been able to get the pieces to unite. It does not seem altogether improbable that the result could be brought about by cutting two stentors at the same time, one about the other. A lucky cut might bring two exposed inner surfaces together, and they might stick to each other, but so far I have not been able to carry out successfully this experiment.

In a few cases the stentor was split partially in two pieces, but generally the halves soon fuse together. It is of some interest to find that, although the peristome was cut in two and had reunited, a new peristome was not produced, showing that the operation alone does not initiate the changes that lead to the development of a new peristome.

The development of a new peristome in a piece that contains a part of the old one appears to be due to the lack of proportion between the old part (even when it contains all the essential parts of the peristome) and the rest of the piece. This result is unique, since in all other forms in which a part of an old organ remains the new organ regenerates from that part. In stentor this does not occur, but a new organ is produced. It is important to observe, however, that this is the characteristic way in which stentor produces a peristome, so that the organisms make use of a process that already exists. The reduction in size of the old peristome in pieces from the anterior end is the result that has most interested me. It seems to be due to the withdrawal of material from the anterior region to form the body and stalk of the new stentor.

The change in shape of the piece, *i.e.*, the production of the typical form, is primarily the result of a shifting of the material that carries with it a loss of material in the old part. Other so-called formative factors may have some share in the reduction in size of the old peristome to one proportionate to the rest of the piece, but the simple loss of material will, I think, account for the greater part of the change.

What primarily brings about this change in the material so that the typical form is produced is a question to which at present there is no answer.

BRYN MAWR, March 12, 1901.

Reprinted from the American Journal of Physiology.
VOL. V. — JULY 1, 1901. — NO. VI.

ON THE FORCE OF CONTRACTION OF THE FROG'S GASTROCNEMIUS IN RIGOR, AND ON THE INFLUENCE OF "CHLORETONE" ON THAT PROCESS.

BY N. M. STEVENS.

[*From the Physiological Laboratory of Bryn Mawr College.*]

IN the course of a series of experiments involving rigor of the frog's gastrocnemius, performed during the autumn of 1900, in the Physiological Laboratory of Bryn Mawr College, under the direction of Dr. Joseph W. Warren, the writer became impressed with the idea that much greater energy is exerted by a muscle undergoing rigor at a high than at a low temperature, and that the force of contraction varies directly as the temperature. A new series of experiments was therefore performed to test this impression, which was based only upon observation of the rise of the lever during rigor under various conditions.

The absolute energy exerted by the gastrocnemius in heat rigor has been measured by Hermann and Walker,[1] and compared with the energy of single contractions and of tetanus; but, so far as I know, no systematic work has hitherto been done to show the effect of temperature upon the energy of rigor contraction.

METHOD.

Apparatus. — The apparatus (Fig. 1) employed in these experiments with the exception of the spring, has been used in this laboratory for similar work since 1895, but has not been described.

It consists of a simple kymograph, a muscle-holder, and a reservoir of tin (an ordinary washboiler) supported on stands, beneath which gas burners may be placed as required.

The kymograph has a brass recording drum and spindle, A, set in the frame, B, and turned by a Seth Thomas eight-day marine clock sliding on a wooden track and fastened by a thumb-screw in any desired position. The minute and hour hands are replaced by two hin brass discs, one inch in diameter, and grooved for the waxed cord, K. The cone, C, composed of four circular plates of hard rubber, 1, 2, 3, and 4 inches in diameter respectively, cemented together on a central brass tube, slips up and down on its spindle and is fastened in position by the pin, D. By changing the cord from the minute to the hour wheel of the clock, and from one to another groove of the cone, adjusting the height of the cone for each change, the drum is made to revolve in 1, 2, 3, 4, 12, 24, 36, or 48 hours. The upper end of the spindle terminates in a circular plate, E, on which the drum is carried in the usual manner. The foundation of the structure is a hard wood board 20 x 48 cm. to which is fastened the clock track and another board, 20 x 21 cm. which carries the drum and marker. The latter part of the apparatus is removable for use in other combinations. The clock is protected from dust by a tin cover, G, through an opening in which the cord, R, passes. The kymograph rests on a stand made from a lecture room desk. In the sketch this stand has been omitted and the depth of the washboiler reduced about half.

---

[1] HERMANN and WALKER: Archiv für die gesammte Physiologie, 1871, iv, p. 182.

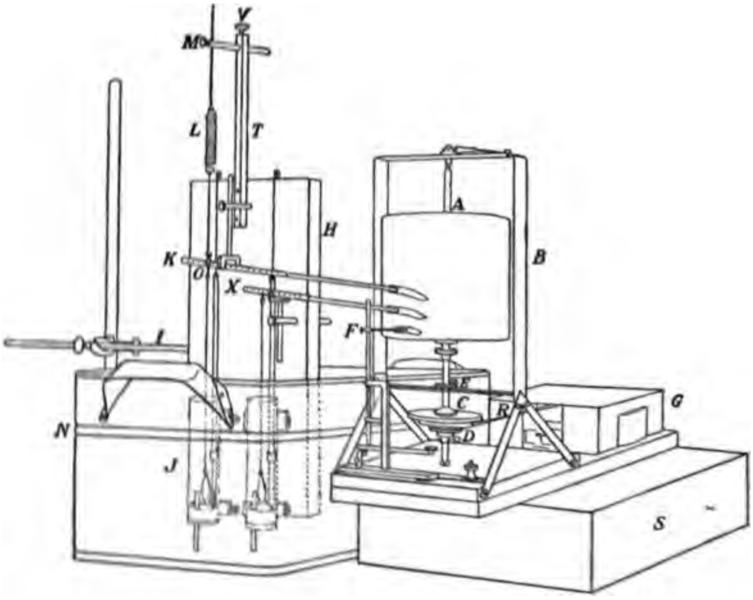


FIGURE 1.

The muscle holder, H, consists of a board, 17 x 26 cm., held in a vertical position by the rod, I, which is clamped into a very firm stand, the base being filled with lead. The muscle cylinders, J, are of glass tubing, about 12 cm. long and 3.5 cm. inner diameter, supported against pieces of paraffined cork grooved to fit the tubing; they are held securely in place by tapes run through screw eyes and firmly tied over the cylinders. Each cylinder is fitted with a rubber stopper through which passes a short piece of paraffined doweling, held in place by a pin below the stopper; the femur of the muscle preparation is fastened into a hole in this rod by the pointed end of a hardwood toothpick.

The muscle is connected with the lever, K, by a light metal rod,[1] platinum below and steel above. This rod hooks into the tendon and into either the loop, O, or one of the holes in the lever.

The levers are of aluminium, pierced with holes at intervals of one fourth of an inch, for the attachment of the muscle-rod and weights, and lengthened by straws terminating in celluloid markers. The drawing (Fig. 1) shows the manner of suspending and adjusting the levers. The magnification is ten times, the long arms of the levers being 10 and 7.5 inches, the short arms 1 and ¾ inches, respectively.

For the attachment of the spring, L, a brass pillar, T, was screwed to the board. Near the top of the pillar is a brass rod, projecting at right angles to the surface of the board, and adjustable by the set screw, V. Through this rod passes a thick steel wire, to which is suspended a somewhat stiff steel spring of twenty-five turns. The spring is connected with the lever by another steel rod which hooks into a light brass support pinned through the hole in the lever with which the muscle is connected. The spring stretches just enough to let the lever make two distinct parallel lines on the drum for a difference of one gram in a scale pan hung from the loop, O. The rise of the lever for a weight of 200 grams is about half of the rise for the ordinary unresisted heat contraction of a muscle 30 mm. long.

---

[1] Paraffined or waxed threads were formerly used for such experiments, but certain peculiarities in some of the curves led me to think that even the most careful paraffining or waxing of the thread was not a sufficient safeguard against shrinkage. A quantity of linen thread was, therefore, thoroughly shrunken for immediate use, and later the matter was carefully investigated by setting up paraffined and waxed threads of linen, cotton, or silk, in liquid and in the cylinders used as moist chambers. In every case there was shrinkage for three or four hours, the temperature being raised, meanwhile, from 17° to 70° or thereabouts; upon cooling down to room temperature, the threads lengthened somewhat and shortened again slightly on heating, even after standing forty-eight hours, the final result being a shortening of 3.6 mm. for the length used in this apparatus, about 24 cm. A cotton thread shortened 5.3 mm. in three hours between 17° and 68°, lengthened 2.5 mm. during slow cooling to 17°, and again shortened 1 mm. on renewed warming about twenty hours after the first heating, giving a final shortening of 3.8 mm. Linen thread which had been soaked alternately in hot and cold water for several hours and then dried, showed no marked shrinkage when heated in the Ringer solution.

Vernon's statement (Journal of physiology, 1899, xxiv, p. 239) of the unreliability of thread for such work came to hand after the first tests were made, and his use of a metal chain was noted; but the rods, already in use for electrical experiments, seemed more convenient to handle, and were immediately substituted for the thread.

A thermometer hung from a screw-eye in the top of the board, enters each cylinder, and pieces of thick rubber tubing hold the bulb away from the glass and near the muscle.

The reservoir is usually kept filled to the groove, N, the large amount of water, 30–35 litres, protecting the muscles from rapid changes of temperature when this is not desired. The amount of liquid used in the cylinders depends upon its character and upon the character of the experiment: at least enough must be used to cover the muscle and tendon.

Heat is supplied by an argand burner, with an adjustable arm, to this are added as many Bunsen burners as are required for rapid heating.

Preparation of the Muscles. — The frog was pithed, the muscle preparations removed as quickly and carefully as possible, and set up in the cylinders (Fig. 1). The spring, which was left loose while the connection between muscle and lever was being made, was now set at M to straighten the muscle. The liquid to be used was then poured into the cylinders, the levers adjusted to the drum, and heat applied to secure the desired temperature. The left gastrocnemius was used on the spring in each case, and the right as a control.

Every effort was made to avoid the use of any material which, in connection with the liquids employed, might injure the muscle. No metal except platinum was brought into contact with muscle or liquid; the rubber stoppers were thoroughly scalded and soaked for twenty-four hours before using; the platinum rods were sterilized in the flame before each experiment; and the cylinders were frequently scalded, especial care being taken in case of experiments continuing many hours.

In the first series of experiments a Ringer solution according to the following formula (Rusch) was used: —

Distilled water.	1000 c.c.
$NaHCO_3$	0.1 gm.
$CaCl_2$.	0.1 gm.
KCl	0.075 gm.
NaCl	8.000 gm.

The results of ten experiments are given in Table I. In the temperature column, the first number given in each case shows the temperature at the beginning of the experiment; the second number, in heavy type, the temperature at the close of contraction in Experiments 1 to 5, during the whole contraction in Experiments 6 to 8. In Experiment 9 the temperature varied considerably; it ran lower at night, but did not go above 22° C., and stood at that point during contraction. In Experiment 10 the culmination of contraction occurred at about 2 A. M., with the temperature somewhere between 20° and 12°, the latter temperature being observed at 8 A. M.

TABLE I.

Muscles in Ringer's solution.

No.	Length in mm.	Temperature C.	Time.		Force in grams.	Work in gm. mm.
			h.	m.		
1	27	31.0°-**44.0°**	0	20	97.80	127.14
2	27	35.0°-**44.5°**	0	25	99.30	131.57
3	30	35.0°-**44.0°**	?		100.00	134.00
4	30	35.0°-**44.0°**	0	15	106.70	149.38
5	30	37.0°-**45.0°**	?		110.00	156.75
6	30	25.0°-**35.0°**	6	20	55.00	41.25
7	32	25.0°-**29.5°**	16	0	27.95	9.08
8	30	22.0°-**27.0°**	29	0	16.50	2.64
9	36	15.5°-**22.0°**	58	0	3.60	0.18
10	32	22.0°-12.0°	63	0	2.00±	0.05±

The pull of the muscle on the spring was determined[1] by ascertain-ing how many grams in a light scale-pan suspended from O (Fig. 1) were required to raise the lever from a base line drawn through the point where the lever began to rise to another line drawn through the highest point in the curve. The work done was calculated by the usual formula for an elastic resistance, $W = F \times \frac{1}{2}E$, E in this case being the resistance of the spring.

---

[1] An attempt was made to establish a constant for the spring, but though C was found to be very nearly 40 grams to a millimetre, the method employed was thought to be more exact.

The muscles vary so much in thickness as well as in length, that the results can only be comparative, but as such they are sufficiently striking.

The question arose whether this decrease in energy of rigor contraction might not be due in part to changes induced in the muscle by long soaking in the Ringer solution. After some consideration of the various oils which have been used in muscle experiments, a light paraffin oil was selected as a medium which would certainly not be injurious, and would not be likely to be absorbed by the muscle, or to take anything from the muscle. The results of a second series of experiments, using the paraffin oil in place of the Ringer solution, are given in Table II: —

TABLE II.

Muscles in paraffin oil.

No.	Length in mm.	Temperature C.	Time.		Force in grams.	Work in gm. mm.
			h.	m.		
11	28	23°-44.7°	0	40	81.00	85.86
12	31	24°-44.3°	0	35	127.35	213.31
13	30	35.0°	7	0	70.20	64.58
14	30	29°-33.0°	15	0	44.00	26.40
15	29	22°-27.0°	30	0	31.70	18.79
16	34	17°-22.0°	53	0	3.60	0.18

Experiment 11 of this series was a very slender muscle, containing much less substance than Experiments 1 or 2 of Table I, and the muscle of Experiment 13 was very much stouter than that of Experiment 6, the corresponding muscle of Table I.

The curves shown in Fig. 2 were traced directly from the drum record of Experiment 2, those in Fig. 3 from Experiment 12. Both are characteristic curves, but the peculiarities observed in comparing one with the other are not characteristic of experiments with either the Ringer solution or the oil. The counterpart of any variation in form of curve or of temperature limits may be found in the records of experiments with the other liquid. Comparison of the two tables indicates that the results obtained are independent of the liquids used.

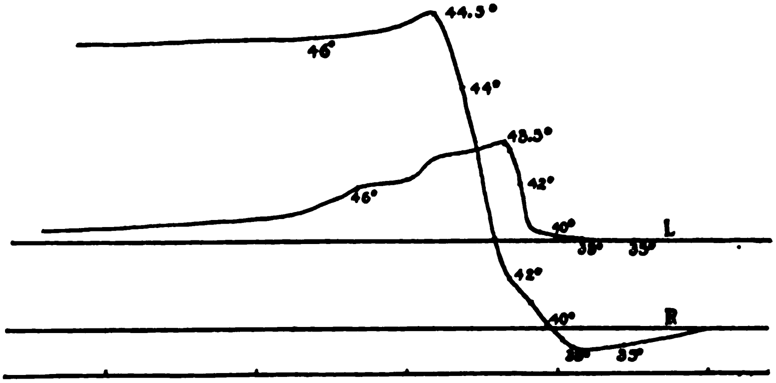


FIGURE 2.

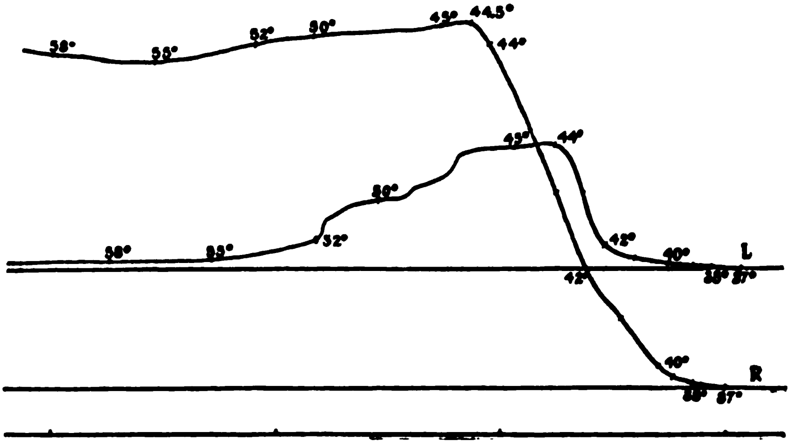


FIGURE 3.

Secondary heat contractions were obtained in the control muscles between 50° and 58°, and between 60° and 63°; but in no case did these contractions exert any additional force on the spring.

The temperature limits in these experiments are higher than those given by Brodie and Richardson,[1] for corresponding heat contractions in the sartorius of the frog, but agree with those given by Latimer[2] for the gastrocnemius.

This difference is probably due mainly to the greater thickness of the gastrocnemius, but the statement of Moriggia[3] that the proteids of winter frogs coagulate less readily may have some bearing on the point.

For comparison with Walker's results, a few experiments were performed, in which one muscle of a pair pulled on a spring, during heat contraction, and the other was "afterloaded" with two hundred grams.

The only change in the apparatus for these experiments was the addition of a firm support for the lever X, — a heavy brass rod, bent at right angles and passed through an adjuster projecting from a metal plate which could be fastened to the board, in any desired position, with an ordinary clamp.

The following table is a record of four such experiments, the work being completed by the usual formulæ, $W = F \times \frac{1}{2} E$, and $W = F \times D$.

TABLE III.

No.	Length in mm.	Spring.		Load of 200 gms. Work in gm. mm.
		Force in grams.	Work in gm. mm.	
1	30.0	142.3	256.14	130
2	29.0	138.9	239.60	90
3	27.5	104.0	140.40	30
4	27.0	98.1	122.60	10±

INFLUENCE OF "CHLORETONE" ON RIGOR CONTRACTION.

The so-called "chloretone" of commerce (Acetonchloroform = trichlor tertiary butyl alcohol) having been found useful for several purposes in this laboratory, it was suggested that its direct action on frog's muscle might prove interesting.

---

[1] BRODIE, T. G., and S. W. RICHARDSON: Philosophical transactions, cxci, p. 127.

[2] LATIMER, CAROLINE W.: This journal, 1898, ii, p. 29.

[3] MORIGGIA: Moleschott's Untersuchungen, 1892, xiv, p. 386.

The apparatus used was the same as in the preceding experiments. The chloretone was dissolved in the same Ringer solution, and 150 c.c. were used for each muscle. The temperature of the water in the reservoir was kept at 30° and that of the chloretone solution, when it was poured into the cylinders, was about 20°. The results were practically the same with the reservoir water at room temperature, but the time required for the experiments was considerably longer.

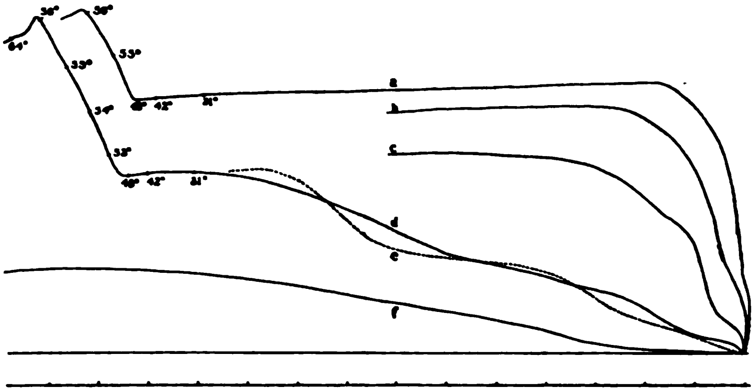


FIGURE 4.

The curves of contraction made by three pairs of muscles in chloretone solutions of different degrees of concentration, 0.1 per cent to 0.5 per cent of chloretone crystals in Ringer solution, are shown in Fig. 4, and further details are given in Table IV.

TABLE IV.

Muscles in Chloretone.

	Length in mm.	Per cent of chloretone.	Time.	Per cent of shortening.
			h. m.	
a	29	0.5	0 58	37.2
b	29	0.4	1 28	34.1
c	26	0.3	3 0	30.7
d	26	0.2	5 40	27.7
e	26	0.2	4 50	28.0
f	26	0.1	6 30	12.7

The levers were adjusted to the drum before the solutions were poured into the cylinders, and all of the muscles except f, at once began to shorten more or less rapidly according to the concentration of the solution; f, however, began to contract only after eight and one-half hours.

The rate and percentage of shortening decrease with the strength of the solution, much as in rigor at different temperatures from 45° to 20°, but with greater regularity and certainty.

On applying heat to muscles that have undergone chloretone contraction, no change is observed between 37° and 45°, but secondary heat contractions, perfectly comparable with those of muscles which have undergone rigor mortis or rigor caloris, occur between 50° and 60°. The height of these contractions depends on that of the previous contraction, as is shown in Fig. 4. The secondary contraction, which is evidently a combination of the second and third heat contraction described by Brodie and Richardson for the sartorius, is enough greater in one muscle of a pair, treated with a 0.2 per cent chloretone solution, than that of the other in a 0.05 per cent solution, to make the entire shortening in the two muscles approximately the same.

The same phenomena are observed in muscles which have undergone rigor mortis at different temperatures: a large secondary heat contraction follows a small rigor mortis contraction, and *vice versa.*

In both cases, the first heat contraction, due, according to von Fürth[1] and to Brodie and Richardson, to coagulation of soluble myogen fibrin, is cut out, in one case by the chloretone contraction, in the other by rigor mortis; but the contractions, due according to the same authors, to coagulation of myosin and myogen, and to contraction of connective tissue occur as usual, but with the variations described above. It would seem that there must be a different molecular disposition of the coagulated substance in a slow primary rigor contraction which makes a greater secondary contraction mechanically possible.

The effect of chloretone in 0.5 per cent solution seems to be quite similar to that described by Kühne[2] for potassium sulphocyanide (Rhodankalium), but its action appears to be definitely selective, precipitating soluble myogen fibrin, and not myosin or myogen (von Fürth).

---

[1] VON FÜRTH: Archiv für experimentelle Pathologie und Pharmakologie, 1895, xxxvi, p. 231, and 1896, xxxvii, p. 389.

[2] KÜHNE: Archiv für Physiologie, 1859, p. 634.

The action of chloretone on the proteid extracts of muscle should now be ascertained: if it should be shown that it precipitates soluble myogen fibrin in solution, and not myosin or myogen, we should have positive evidence that these proteids exist as such in the living muscle, and that the phenomena of heat rigor are due to fractional coagulation or precipitation of the same proteids.

For comparison with the chloretone contractions, a few experiments were performed with chloroform water (saturated solution), and with potassium sulphocyanide (KSCN), the Rhodankalium of Kühne.

The curves obtained with chloroform water were practically identical with those of chloretone, 0.5 per cent solution. This fact supports the theory that the action of chloretone as an anæsthetic is due to separation of chloroform in the tissues.

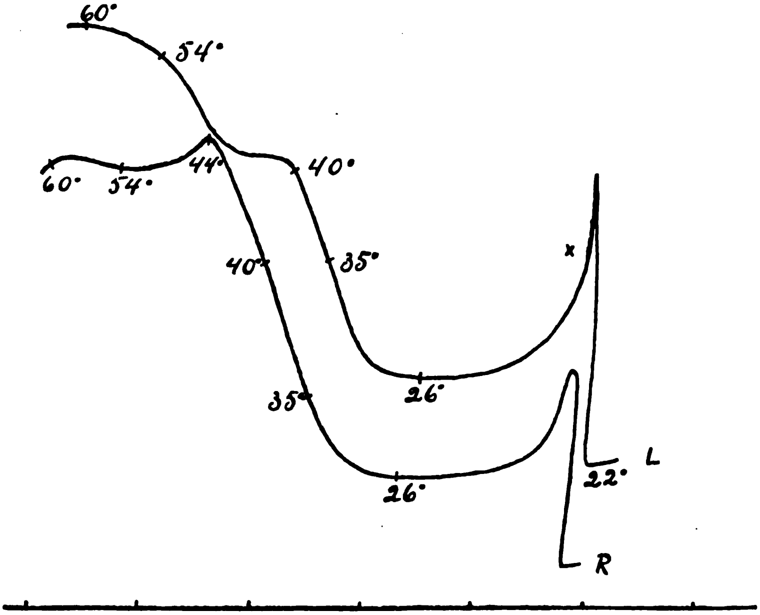


FIGURE 5.

The curves produced by the action of the potassium sulphocyanide showed unexpected peculiarities. At first, a 1 per cent solution as mentioned by various authors, was used, and later ½ per cent and 2 per cent solutions.

In every case the lever rose rapidly to a certain variable height and then sank, the amount of rise and fall varying with the strength of the solution. After the lever ceased to fall, heat was applied and more or less irregular primary and secondary heat contractions occurred.

Fig. 5 shows the curves made by a pair of muscles, L in a 2 per cent solution, R in a ½ per cent solution. At X the muscle gave several slight twitches, showing that it had not been deprived of irritability by the sulphocyanide. The whole appearance of the initial contraction suggested a sort of tetanus. One pair was taken down after an hour without heating, and the muscles appeared perfectly fresh: another pair was left set up for several hours at room temperature: within an hour from the beginning of the experiment, both of these muscles made slight contractions and then gradually lengthened; after forty-one hours both muscles made large contractions on heating to between 50° and 60°.

Four different samples of the chemical, all supposed to be "C. P.," were tried with the same result.

The results of these last experiments, compared with those performed with chloretone tend to confirm the impression that chloretone may prove valuable in the study of proteid coagulation in its relation to rigor contraction.

CONCLUSIONS.

1. The energy of rigor contraction decreases rapidly with decrease in temperature, being very slight at 20° C. or below.

2. The energy of secondary heat contractions (50°–63°) in the gastrocnemius is less than that of ordinary rigor contraction at 20° C.: it is not measureable by the spring used in these experiments, even when the muscle has been stretched to its original length before the temperature of secondary heat contractions is reached.

3. There is a certain degree of parallelism between temperature, rate, amount, and energy of rigor contraction.

4. A gastrocnemius muscle, undergoing heat rigor, does more work when it contracts against a spring than when it is "after-loaded" (200 grams.)

5. The fact that complete rigor at 20° C. entirely cuts out the first heat contraction (37°–45°), but not the secondary heat contractions (50°–63°) indicates that rigor mortis of frog's muscle involves coagulation of soluble myogen fibrin (v. Fürth), but not of myosin or myogen.

6. The fact that immersion of a muscle in chloretone solution

(0.1–0.5 per cent) produces a contraction which cuts out the first heat contraction (37°–45°), but not the secondary contractions (50°–63°) indicates that the result of its action is coagulation of soluble myogen fibrin, but not of myosin or myogen (v. Fürth).

7. A slight contraction produced by weak chloretone solutions (0.1–0.3 per cent) is compensated by a proportionately greater secondary heat contraction, — as in the case of slight rigor mortis contractions (Brodie and Richardson), — indicating a different molecular disposition of the coagulated proteid in a slow primary contraction, making a greater secondary heat contraction mechanically possible.

8. The action of chloretone is very definite and constant, as compared with that of potassium sulphocyanide.

In conclusion, I desire to express my thanks to Dr. Joseph W. Warren for suggestions and criticism.

Observations and Experiments on Regeneration in Hydra viridis.

By

Dr. Helen Dean King.

With 31 figures in text.

Eingegangen am 24. Mai 1901.

During the past three years, several papers have appeared dealing with regeneration and grafting in various species of Hydra. These papers have, in the main, formed the starting point for the following experiments which were made in the hope that they would throw additional light on the processes of regeneration and regulation in this form. The work was carried on during the winter of 1900—1901, under the direction of Professor T. H. MORGAN to whom I am indebted for helpful advice and criticism.

I. Material and Method.

The small green polyp, Hydra viridis, was used in all the experiments as it could be obtained in great numbers throughout the winter months, and also because it regenerates more quickly, and seems to survive more severe operations than does the larger form, Hydra grisea.

A shallow dish with a thin layer of paraffine in the bottom was used in making all the experiments. The dish nearly full of fresh water, was placed on the stage of a dissecting microscope and the animals to be operated upon were then transferred to it. The polyps very soon attached themselves to the paraffine, and cuts were then made with a small sharp scalpel in any desired direction.

In all the grafting experiments a fine glass thread was used to hold the parts of the graft together, as bristles or hairs when delicate enough for this purpose are very flexible and not readily pushed through the tissues without lacerating them. By heating small glass tubing over the blowpipe flame it is easily drawn out into a thread which can be made very fine and still firm enough to pass readily through the tissues. The glass thread was always left attached to a piece of the larger tubing which served as a handle in grafting thus making the use of forceps unnecessary. After the pieces of hydra had been brought together, the part of the glass thread containing the graft was broken off from the handle and one end stuck obliquely into the paraffine. The graft was therefore surrounded on all sides by water, and, if the parts had been brought in close contact in the beginning of the experiment, they so rarely separated that at least nine tenths of all the grafts were successful. In from one to two hours the components of the graft were firmly united. The glass rod was then removed and the graft transferred into a small glass dish containing fresh spring water. Green water plants (Nitella, Cara etc.) were always kept in the dishes containing the hydras and small Crustacea (Daphnea, Cyclops etc.) and rich cultures of Paramoecia (obtained from hay infusions) were supplied as food. The polyps were not attacked by fungus growths, or bacteria; and many of them were kept in good condition for over three months, during which time they gave off many buds and in some cases produced ova.

II. Relation between the Size of a Hydra and the Number of Tentacles it Bears.

In his recent paper dealing with the »Variation and Regulation of Abnormalities in Hydra«, PARKE (12) has stated that his observations seem to show that the size of a hydra is directly proportional to the number of tentacles it bears; that if this ratio is altered by an increase or a decrease in the size of the hydra, there will be a corresponding increase or decrease in the number of tentacles. The following experiment was made to determine, if possible, whether such a relation exists in all cases, and if so, how it is brought about.

A number of polyps were taken at random from several aquaria and separated into groups according to the number of tentacles each bore, no attention being paid to the size of the hydra. The head of each individual was then cut off close behind the circle of tentacles, and the posterior and anterior pieces of each sort carefully isolated.

If PARKE's view is correct not only ought each posterior piece of hydra, which is, of course, of less volume than the original individual, to regenerate fewer tentacles than were removed; but each of the small hydras, produced from an anterior piece, should absorb as many of its tentacles as would necessary to bring about the proper relation between the size of the individual and the number of its tentacles. The body of a hydra which originally had eight or nine tentacles would be expected to regenerate more tentacles than one with originally but six or seven tentacles; and a hydra-head having originally eight or nine tentacles ought, on this theory, to lose more of its tentacles than one which had but six or seven tentacles in the beginning.

The following table shows the number of tentacles which had regenerated on the posterior pieces fourteen days after the operation.

Table I.

Originally six-tentacled Hydras		Originally seven-tentacled Hydras	
No. individuals	No. tentacles regenerated	No. individuals	No. tentacles regenerated
5	6	8	7
6	5	10	6
1	8	2	5
Total 12	68	20	126

Originally eight-tentacled Hydras		Originally nine-tentacled Hydras	
No. individuals	No. tentacles regenerated	No. individuals	No. tentacles regenerated
2	8	2	8
9	7	1	7
3	6	1	6
2	5		
Total 16	107	4	29

Average number of tentacles regenerated by six-tentacled hydras 5.6
- - - - - - seven-tentacled - 6.3
- - - - - - eight-tentacled - 6.6
- - - - - - nine-tentacled - 7.1

In all cases except one of the six-tentacled hydras which for some unknown reason produced eight tentacles, fewer tentacles were regenerated than were cut off in the beginning of the experiment, and the individuals having originally the greatest number of tentacles regenerated the greatest average number. These results are in full accord with those of similar experiments made by RAND (15) to determine the relation between the original number of tentacles in an individual and the number of tentacles regenerated, and seem to verify PARKE's conclusion that a direct proportion exists between the size of an individual and the number of tentacles it bears.

The anterior pieces of hydra formed small polyps in the course of three or four days, and many of them lived for over two months. In not a single case was there a decrease in the number of tentacles even in the polyps which had originally nine tentacles; on the contrary, the number of tentacles was increased by one or two in several instances. The hydras formed from these small anterior pieces could not have been more than one fourth of the volume of the original individuals, yet no attempt was made on the part of the polyp to decrease the number of its tentacles so as to establish any definite relation between the size of the hydra and the number of its tentacles. This result does not support PARKE's view and seems to show that there is some important factor other than the size of the hydra which determines the number of tentacles in any given case.

The size of the hydra is undoubtedly one of the important factors in determining the number of tentacles which will regenerate on the hypostome of the new individual formed from a piece of the body wall, as the results of Table I show. PEEBLES (13) has found that the smallest piece of hydra capable of regeneration never forms more than one tentacle, while a piece slightly larger is able to regenerate but two tentacles. RAND has recorded the results of an extensive series of experiments which seem to show that the number of tentacles that regenerates depends, to a great extent, on the size of the hydra. If, however, a given number of tentacles is already present, the last experiment shows that there is no necessity for the number to be changed in order to establish a definite ratio between the size of the individual and the number of its tentacles. If is very probable that in the cases observed by PARKE in which the number of tentacles was decreased that the polyps were not in good condition. If the water in which the hydras live is allowed to become stale, or if food is lacking the hydras invariable become much smaller, the number of tentacles decrease and the polyps soon die.

Is the size of the hypostome also a factor in determining the number of tentacles which a polyp bears? To test this point a number of hydras were allowed to become fully expanded and the head of each was cut off as closely as possibly behind the circle of tentacles. The diameter off each hypostome was then measured under a Zeiss AA. ocular 4. The measurement was made when the tentacles were fully expanded and in each case it extented as nearly as possibly across the centre of the hypostome from the inner base of one tentacle to the inner base of another tentacle on the opposite side of the mouth. On account of the constant movements of the polyps it was not possible to make very accurate measurements, but the figures given in the following table are approximately exact.

Table II.

Six-tentacled Hydras		Seven-tentacled Hydras		Eight-tentacled Hydras	
No. individuals	Diameter of hypostome in millimeters	No. individuals	Diameter of hypostome in millimeters	No. individuals	Diameter of hypostome in millimeters
2	0.18 mm	1	0.20 mm	3	0.22 mm
3	0.20 -	5	0.22 -	1	0.24 -
2	0.22 -	1	0.26 -	4	0.26 -
				2	0.28 -
Total 7		7		10	

Nine-tentacled Hydras		Ten-tentacled Hydras	
No. individuals	Diameter of hypostome in millimeters	No. individuals	Diameter of hypostome in millimeters
1	0.28 mm	1	0.32 mm
2	0.30 -	1	0.34 -
2	0.32 -		
Total 5		2	

Average diameter of the hypostome of a six-tentacled hydra. . . . . 0.20 mm
- - - - - - - seven-tentacled hydra . . . 0.22 -
- - - - - - - eight-tentacled - . . . 0.25 -
- - - - - - - nine-tentacled - . . . 0.30 -
- - - - - - - ten-tentacled - . . . 0.33 -

The average difference in the size of the hypostomes of hydras bearing different numbers of tentacles varies between 0.02 mm and 0.03 mm in all cases excepting between the eight and nine-tentacled hydras where for some unknown reason it is increased to 0.05 mm, dropping down again to 0.03 mm between the nine and ten-tentacled hydras.

It is to be regretted that more measurements could not be made of the hypostomes of ten-tentacled hydras, but only two individuals having this number of tentacles could be found among the three or four hundred hydras examined for this purpose. Some search was also necessary to obtain the five specimens of nine-tentacled hydras, but polyps having six, seven or eight tentacles were very abundant. The hydras measured were taken from four different aquaria and as they were selected solely on account of the number of tentacles they were presumably individuals of average size.

The above Table shows that there is considerable individual variation in the size of the hypostomes of polyps bearing the same number of tentacles and that no sharp line can be drawn between the different groups of hydra in this respect, as the measurements overlap in every case. When, however, an average is made of the size of the hypostomes in the different groups, it is seen that normal hydras having the fewest tentacles have also the smallest hypostomes and that there appears to be a direct proportion between the size of the hypostome and the number of tentacles it bears.

The posterior and anterior pieces into which the polyps were divided in order to make the above measurements, were carefully isolated and allowed to regenerate into complete individuals. The question arises as to whether the new hypostome formed on a posterior piece will be as large as that of the polyp before the head was removed or whether it will be decreased in size to correspond with the decrease in the size of the hydra.

One week after the first measurements were made the heads were cut off of the regenerated hydras and measurements made as before. The results are given in the following Table.

Table III.

Originally six-tentacled Hydras			Originally seven-tentacled Hydras		
No. Hydras	No. tentacles regenerated	Diameter of hypostome	No. Hydras	No. tentacles regenerated	Diameter of hypostome
1	4	0.12 mm	2	5	0.16 mm
2	5	0.14 -	3	6	0.18 -
1	5	0.16 -	2	6	0.20 -
1	6	0.16 -			
1	6	0.18 -			
1	6	0.20 -			
Average	5.29	0.15 mm		5.7	0.18 mm

Originally eight-tentacled Hydras			Originally nine-tentacled Hydras		
No. Hydras	No. tentacles regenerated	Diameter of hypostome	No. Hydras	No. tentacles regenerated	Diameter of hypostome
1	4	0.14 mm	1	7	0.18 mm
3	6	0.16 -	1	7	0.22 -
2	6	0.20 -	2	8	0.20 -
1	7	0.20 -	1	8	0.22 -
3	7	0.22 -			
Average	6.2	0.19 mm		7.6	0.20 mm

Originally ten-tentacled Hydras		
No. Hydras	No. tentacles regenerated	Diameter of hypostome
2	8	0.22 mm
Average	8	0.22 mm

From the above table it is seen that the polyps with originally the smallest number of tentacles have regenerated the smallest average number. The smallest hypostomes are found on the polyps with the fewest tentacles, so that there is the same direct proportion between the size of the hypostome and the number of tentacles that is shown in Table II. The average diameter of the hypostome of a normal six-tentacled hydra is 0.2 mm, but that of the regenerated polyp is but 0.15 mm, a difference of 0.05 mm. The difference is 0.04 mm in the seven-tentacled hydras; 0.06 mm in the eight-tentacled hydras; a marked increase to 0.10 mm in the case of the nine-tentacled polyps; and a further increase in the ten-tentacled hydras to 0.11 mm. In the first three cases the difference is well within the limits of individual variation which in the eight-tentacled hydras is as much as 0.06 mm; but the great increase in the nine and ten-tentacled hydras is far in excess of this, so that it is not probable that individual variation can account satisfactorily for the facts in any case.

Morgan (10) has called attention to the fact that the new polyp formed from a piece of the body wall of a hydra is produced by a remodelling of the old material without the apparent formation of any new tissue. The size of the polyp thus formed depends on the size of the original piece of body wall. The hypostome formed on the new individual would naturally be smaller than that of the original polyp in order to correspond with the size of the new hydra. That a hypostome when already present is also remodelled to correspond with the size of the polyp is shown by the following measurements of the hypostomes of the small polyps formed from the heads of the hydras cut off in the previous experiment. The measurements were made at the same time as those recorded in Table III.

Table IV.

Six-tentacled Hydras		Seven-tentacled Hydras		Eight-tentacled Hydras	
No. Hydras	Diameter of hypostome	No. Hydras	Diameter of hypostome	No. Hydras	Diameter of hypostome
2	0.14 mm	1	0.16 mm	2	0.18 mm
4	0.16 -	4	0.18 -	6	0.20 -
1	0.18 -	2	0.20 -	2	0.22 -
Average	0.157 mm		0.18 mm		0.20 mm

Nine-tentacled Hydras		Ten-tentacled Hydras	
No. Hydras	Diameter of hypostome	No. Hydras	Diameter of hypostome
2	0.24 mm	1	0.26 mm
2	0.26 -	1	0.30 -
1	0.28 -		
Average	0.25 mm		0.28 mm

Loss in diameter of the hypostomes of the six-tentacled hydras . . . 0.05 mm
- - - - - - - - - seven-tentacled hydras . . 0.04 -
- - - - - - - - - eight-tentacled - . . 0.05 -
- - - - - - - - - nine-tentacled - . . 0.05 -
- - - - - - - - - ten-tentacled - . . 0.05 -

Although in no case has the number of tentacles been diminished, yet the average diameter of the hypostomes has decreased about 0.05 mm, irrespective of the number of tentacles possessed by the polyp. The loss can be well accounted for on the ground that the hypostome has been remodelled in each case to accord with the smaller size of the new polyp. The remodelling of the hypostome does not in any way affect the number of tentacles present. It would be interesting if comparative measurements could be made of the lengths of the tentacles of a normal hydra and of one remodelled from a piece of the body wall. The volume of the tentacles, if not the number, may be decreased to correspond with the volume of the new polyp so that a definite ratio is established between the volume of the hydra body and the volume of its tentacles.

Summarizing the results of Tables II, III, and IV, it is seen that the normal hydras with the largest number of tentacles have the argest average hypostomes although individual measurements overlap in many cases; that in polyps remodelled from a piece of the body wall the new hypostome is smaller than that of the original polyp and fewer tentacles are regenerated; that the hypostomes of all the small hydras produced from circles of tentacles decrease in size about 0.05 mm, although there is no corresponding decrease in the number of tentacles.

The size of the hypostome undoubted depends to a great extent on the size of the polyp, and both are probably important factors in determining the number of tentacles that will regenerate in any given case.

If all the tentacles are removed without decreasing the volume of the body of the hydra or the size of the hypostome, will the same number of tentacles regenerate or not? Polyps were held firmly with a blunt needle under a dissecting lens and the tentacles cut off close to the hypostome. If any of the body wall was removed, or if a considerable portion of the base of a tentacle was found to be present when the hydra became extended, the polyp was excluded from the experiment. Fifty individuals were operated upon, but all except 27 were rejected for one or the other of the reasons given above. The following record was made ten days after the operation.

Table V.

Originally seven-tentacled Hydras		Originally eight-tentacled Hydras	
No. individuals	No. tentacles regenerated	No. individuals	No. tentacles regenerated
2	6	8	8
10	7	6	7
		1	6
Total 12		15	

In the majority of hydras the same number of tentacles regenerated as were removed. The cases where fewer tentacles regenerated than were removed may, perhaps, be due to the fact that a small piece of the hypostome was cut off with the tentacles in spite of the care taken to include in the experiment only the individuals in which the tentacles alone appeared to be removed. In no case were more tentacles regenerated than had been cut off, so the mere cutting of the hypostome does not give a stimulus to the production of additional tentacles.

Rand cut off the heads of a number of six and seven-tentacled hydras and then divided the body transversely as nearly as possible into two equal parts. He found that the posterior pieces regenerated a greater average number of tentacles than the anterior pieces. **Rand** attemps no other explanation of the phenomenon than to state that »the excess in the mean for the lower halves is due to the same causes that determined the difference in the original number of tentacles, rather than to a difference in the amount of wounded surface«.

I repeated the experiment using seven-tentacled hydras in all cases. The result agrees entirely with that obtained by **Rand** as the following table shows.

Table VI.

Seven-tentacled Hydras.

No. upper pieces	No. lower pieces	No. tentacles regenerated
—	2	8
3	5	7
4	6	6
7	4	5
5	4	4
—	2	3
2	—	2
Total 23	23	

Average number of tentacles produced by the upper halves 4.5
- - - - - - - lower - 5.6

A difference in the volumes of the upper and lower pieces can hardly account for the uniform result obtained by **Rand** and myself. It is, of course, impossible to make the cut exactly in the middle of each polyp in every case; but the upper pieces are as liable as the lower to be the larger part. Cutting the body transversely through the middle brings the region of the budding zone at the anterior end of the lower piece, and the reproductive zone at the anterior end of the upper piece. This may account for the difference in the number of tentacles produced in the two cases as the region of the budding zone is evidently much more active in regenerating tentacles than the reproductive zone, although the latter lies nearer the oral end of the hydra.

III. The Regeneration of Polyps Split Longitudinally Through a Part of the Body.

TREMBLEY (17) in his experiments on hydra, cut the body of a polyp longitudinally from the oral end to the middle of the body and a few days later obtained a double-headed hydra. He then split each of the two heads in the same manner and obtained a polyp with four heads on one body. He even succeeded in obtaining an eight-headed form by splitting each of the heads of a four-headed polyp. TREMBLEY also states in his memoir that hydra with two, four, or eight feet can be obtained by a similar splitting of the aboral instead of the oral end of a polyp.

I have repeated some of TREMBLEY's experiments and also extended them in order to answer, if possible, some of the following questions: Will each of the two heads formed on a polyp by the method described above, have as many tentacles as the original hydra, or, if not, will the number of tentacles on the new heads bear any definite relation to the number of tentacles on the old head? Will the two heads grow together again at a later period and produce a typical hydra or will they separate and produce two individuals? Is it possible to produce heteromorphosis by this means?

a. Double-headed Hydras.

When polyps are split longitudinally a greater or less distance through either the oral or the aboral end, there is always a great tendency for the parts to reunite at once. This reunion can be prevented in a majority of cases if the cut edges are separated every few minutes for an hour or two after the operation, as only freshly cut surfaces unite readily. The cut edges on each side close in very soon after the operation and perfect cylinders are formed inside of twenty-four hours.

The following table gives a summary of two sets of experiments, one made on Dec. 5th, 1900, the other on Jan. 9th, 1901, in which the oral end of a number of polyps was split longitudinally through the middle line to within a short distance of the foot. The observations recorded in *b* were made two weeks after the operation in each set of experiments.

Table VII.

a.

No. individuals	Original no. of tentacles	24 hrs. after operation No. tentacles on one head	on the other head
6	9	5	4
2	9	6	3
17	8	4	4
9	8	5	3
25	7	4	3
3	7	5	2
6	6	3	3
2	6	4	2
Total 70	524	296	228

b.

No. individuals	2 weeks after operation No. tentacles on one head	on the other head
3	10	7
2	10	6
4	9	4
5	8	6
3	8	5
8	7	7
13	7	6
10	7	5
7	6	6
8	6	5
2	6	4
4	5	5
1	5	4
Total 70	494	392

The 70 individuals had originally a total of 524 tentacles. Two weeks after the operation the polyps had a total of 886 tentacles, there being an increase of 362 tentacles or an average of 5.1 tentacles for each hydra. In two polyps, 10 tentacles were found on one of the new heads, one more than the highest number of tentacles possessed by any hydra before the operation. The greatest increase in the number of tentacles on any one head was four, and in every case there was an increase of at least one tentacle on each head.

In a slight variation of this experiment, eight-tentacled hydras only were used. The head of each polyp was cut off close behind the circle of tentacles and the body was then split longitudinally in the median line, half way to the foot. Table VIII shows the result when the cut edges were prevented from uniting so that two heads formed on each polyp. The observations were made two weeks after the operation.

Table VIII.

No. individuals	No. tentacles regenerated on one head	on the other head
4	8	5
3	7	6
6	7	4
3	6	6
2	5	5
2	5	3
Total 20	133	96

The 20 hydras had originally 160 tentacles, a total of 229 regenerated on the double-headed forms in the course of two weeks, being an increase of 69 tentacles, or an average of 3.4 tentacles for each individual. In no case were more tentacles regenerated on any one head than were originally possessed by the hydra.

A comparison of the observations made in Tables VII und VIII shows that in both sets of experiments the splitting of the oral end of the polyps has caused a marked increase in the total number of tentacles over the number originally possessed by the hydras, and that the average increase in the number of tentacles is 1.7 greater for each individual if the circle of tentacles is not removed in the beginning of the experiment before the longitudinal cut is made.

The increase in the number of tentacles in either of the above sets of experiments cannot be due to a stimulus produced by the mere cutting of the tissues since it has already been shown that the removal of all of the tentacles close to the hypostome is not followed by any increase in the number of tentacles that regenerate (Table V).

Driesch (3) has found that if a piece of the stem of Tubularia is separated into two parts by a longitudinal split through the middle line, that the sum of the tentacles formed on both pieces is greater than the number of tentacles found on the stem before the splitting. In discussing this result Driesch states that there is of necessity more oral surface on the two cylinders formed than there was on the original stem. »Nun entstehen die Anlagen der Tentakel bei der

Reparation als Gebilde, die rings um eine Fläche, eben den Mantel des cylindrischen Cönosarks herum vertheilt sind. Es ist daher eine naheliegende Annahme, dass sie in der Art ihrer Vertheilung, sowie hinsichtlich ihrer absoluten Zahl cet. par. durch die absolute Größe dieser Fläche bestimmt sein möchten.« Ist das aber zugegeben, so folgt aus den vorstehenden geometrischen Betrachtungen ohne Weiteres, dass an den beiden Mantelflächen zweier Partialcylinder, die aus einem ursprünglichen Cönosarkcylinder der Tubularia entstanden sind, zusammen mehr Tentakel cet. par. entstehen können, als an letzterem.

I see no reason why this explanation of the increase in the number of tentacles in Tubularia following a longitudinal splitting of the stem is not adequate to account, in part at least, for the same phenomenon in hydra. When the body of a hydra is split longitudinally through the oral end and two heads are formed, the sum of the perimeters of the hypostomes of the two new heads is necessarily greater than that of the original hypostome as DRIESCH has shown for Tubularia, and an increase in the size of the hypostome is usually followed by an increase in the number of tentacles as will be shown later.

Spontaneous cross division of Hydra was observed by TREMBLEY who says: »J'ai vu en effet des Polypes se partager d'eux-mêmes. L'endroit, où ils se sont partagés, s'est peu à peu retréci, jusqu'à ce qu'ensin, un petit mouvement a pu opérer la séparation. Chaque portion est devenue ensuite un Polype complet.« MARSHALL has observed the same phenomenon in two cases of Hydra vulgaris and states also that if Hydra vulgaris is split longitudinally from the mouth to near the foot, that the parts, if they do not grow together, produce two-headed forms which sooner on later divide to form two complete individuals.

PARKE is the only investigator, as far as I know, who has stated that spontaneous longitudinal division of hydra occurs. He, however, has not watched the process from the beginning and his statement is based solely on the fact that he has found two-headed forms which later separate into two individuals. As an injury to the head, possibly the bite of an enemy, might easily have caused the formation of a second head, I do not think that PARKE is warranted in assuming that the double-headed forms he found must have originated from spontaneous longitudinal division. I have found several double-headed hydras and many other abnormal forms among the stock hydras in different aquaria, but I consider that they have all been produced by some injury.

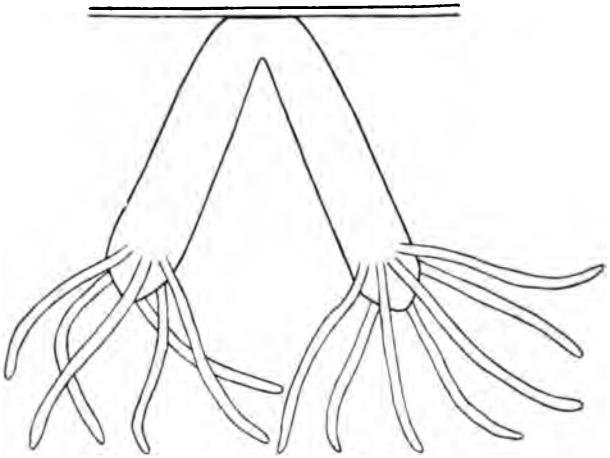
Rand (16) found that when he grafted a piece of a hydra containing tentacles into the side of another hydra, that »there is a slow migration of the graft down the trunk to the foot of the stock«. A constriction then appears and the two pieces separate sooner or later. In discussing this phenomenon Rand says: »It is possible that the migration of the graft is to be explained as a purely mechanical effect of gravity. The much vacuolated cells of Hydra form very watery tissues, which must yield readily to tension or pressure. The weight of the graft is supported at the junction of graft and stock. This weight may be sufficient to cause the cells of the graft to slowly sink downward through those of the stock.«

Is gravity, as Rand suggests, the cause of the separation of the graft and stock in his experiments and also the reason for the separation of the parts of the two-headed hydras observed by Marshall and Parke? To determine this point if possible, 20 hydras were split longitudinally in the median line not quite half way through the length of the body on Jan. 9th, 1901. The next day the 16 double-headed polyps formed were transferred to a glass slide and allowed to become firmly attached. The slide was then quickly inverted and put into a large dish of water, where it rested a short distance below the surface on two cords stretched loosely across the top of the dish and fastened securely on either side. The 16 polyps were thus fixed in an inverted position and gravity, if it has any action on them, must bring the parts together instead of separating them.

In the course of a few days all but one of the polyps dropped from slide and became attached in an upright position to the bottom of the dish. No change could be detected in the hydra which remained attached to the slide in an inverted position until Jan. 21st, when the two heads seemed to be further separated. During this time the two heads were never seen to hang down straight in the water, close together. They were always separated from each other as far as possible, thus causing a constant strain at the place where the two heads joined the body. On Jan. 24th, there was no longer any doubt but that a further separation had taken place as the two heads were seen to be attached only by the foot region (Fig. 1) while the original cut had extended not quite half way through the body of the polyp. On Jan. 26th, the hydra dropped from the slide but became attached to a piece of Nitella in the same inverted position it had occupied on the slide. On Jan. 30th, the two parts were attached only by a very narrow piece of ectoderm in the foot region and four days later the two polyps separated entirely forming two normal individuals, one with six, the other with seven tentacles.

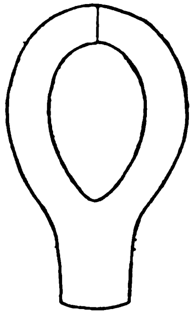
The double-headed hydras which had dropped from the slide and become attached in an upright position were also watched during this period. In one case the two heads separated completely on Jan. 30th, four days before the separation of the parts of the inverted hydra. In 10 other polyps the parts separated in the course of the following three or four weeks, and in 4 cases the heads were still attached three months after the operation. The amount of time required for the heads to separate depends entirely on the extend of the longitudinal cut. If the cut is very shallow and extends only through the hypostome several months may be required for the two heads to separate but complete separation may be effected in the course of a few weeks if the cut extends at least half way through the body of the hydra. In no case have I ever found that two separate heads grew together to form one normal individual, regulation to the normal form is always brought about by a separation of the parts of the polyp into two individuals.

Fig. 1.



The above experiment of fixing a polyp in an inverted position after splitting the oral end half way through the body was repeated later with the same result as in the previous case. These experiments prove conclusively that gravity cannot be the cause of the separation of the two heads in the above experiment nor in the cases observed by Parke and Marshall, and presumably it is not the true explanation of the separation of the graft from the stock observed by Rand. To offer an entirely satisfactory explanation of the phenomenon seems hardly possible. I can only suggest that it may be due to the constant struggle of each part to free itself from the attachment and maintain its own individuality. In all the double-headed polyps, whether they are inverted or not, the two heads always keep as far apart as possible. This cannot help but cause a constant strain on the place of union and it does not seem at all unlikely that the final separation may be caused by this constant effort of the two parts to separate from each other.

Fig. 2.



An interesting abnormal form was obtained in one of the preceding experiments in which the oral end of a polyp had been removed close behind the circle of tentacles and the body then split longitudinally about half way to the foot. Instead if the cut edges uniting to form a normal polyp, or instead of a double-headed form being produced, the cut oral ends fused forming a complete ring (Fig. 2).

Fig. 3.

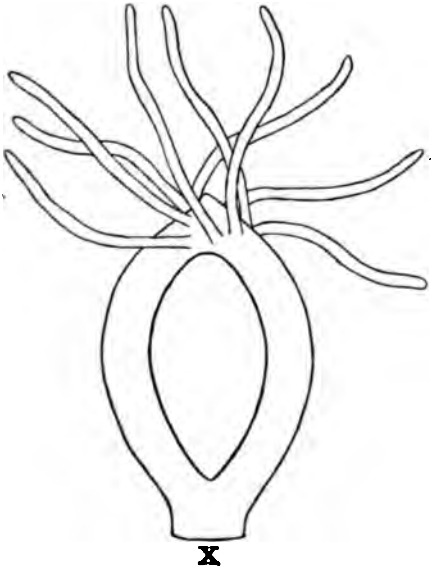
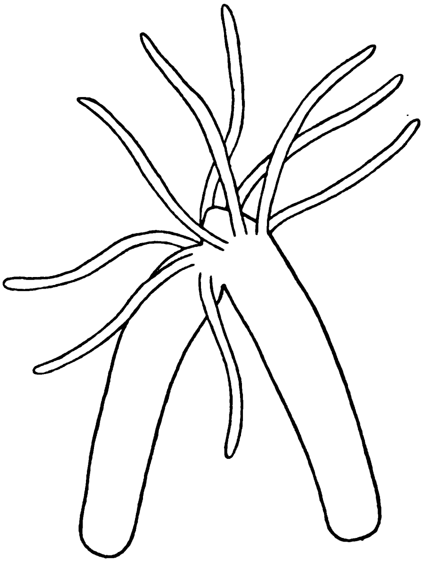


Fig. 4.



Three days later the line of fusion of the cut edges could no longer be seen and four tentacles had appeared in this region. During the next three days the polyp elongated considerably, a hypostome and five more tentacles appeared in the median line and the connection between the two side pieces at the foot became much shorter as shown in Fig. 3. Two weeks after the operation the foot split through the middle (Fig. 3 *X*), and the hydra then appeared as in Fig. 4. The hypostome which had developed in the median line, was seen to belong to the larger piece and the smaller part of the polyp attached by its oral end to the side of the other piece was in the process of constricting off. Separation of the two parts of the hydra was effected at the end of the following week. The smaller piece received three of the nine tentacles. It soon developed a hypostome and became a complete individual.

b. The Reunion of the Cut Edges After a Longitudinal Splitting of the Oral End of a Hydra.

In spite of the repeated separation of the cut edges during an hour or more after the operations recorded in the previous section, the parts reunited in about one fourth of the individuals. When this reunion takes place is there any change in the number of the tentacles as a result of the operation? Table IX gives the results when the cut edges reunited after a longitudinal cut through the oral end of the hydra without a previous removal of the circle of tentacles. The hydras used in the experiment had from six to eight tentacles each. The observations were made two weeks after the operation.

Table IX.

No. individuals	No. tentacles regenerated
2	8
6	9
2	10
3	11
4	12
3	13
2	14
Total 22	238

The 22 hydras had a total of 238 tentacles two weeks after the splitting of the oral end. This is an average of 10.8 tentacles for each polyp.

The cut edges reunite in the course of a few hours after the experiment is made, and there is evidently a rapid growth of new tissue in the injured region during the next few days. If the operation has been made on a very dark green polyp, there is sometimes found, for three or four days after the operation, a wedge shaped piece of tissue of a much lighter green in color in the region where the wound has healed. This is doubtless new tissue which has been formed as a result of the injury and its formation has, of course, increased the volume of the whole hydra as well as the size of the hypostome, to which facts the increase in the number of tentacles is probably to be attributed. The new tentacles which are formed invariably come in on the arc of the hypostome where the cut edges have united, they do not come in between the bases of the old tentacles (Fig. 5). When the circle of tentacles (eight in number) was removed and the body split longitudinally, the number of tentacles found on the head of each polyp two weeks after the cut edges had reunited is shown by the following Table.

Fig. 5.

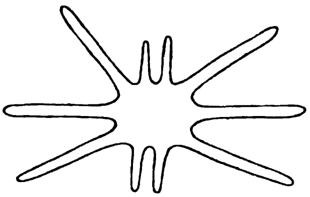


Table X.

No. individuals	No. tentacles regenerated
3	7
7	8
7	9
2	11
1	12
Total 20	174

The 20 hydras have regenerated 174 tentacles, 14 more than the total number originally possessed by the polyps before the oral end was removed. There is also in this case an increase in the total number of tentacles, but the average increase per hydra is only 0.7, this being at least 2.1 less than the increase in the experiments recorded in Table IX. This difference is undoubtedly to be attributed to the fact that the heads of the hydras were cut off in the latter experiments before the longitudinal cut was made, and the amount of new tissue formed in the region of injury is not sufficient to cause as great an increase in the size of the hypostome as in the former case.

Comparing the records of Table VIII with those of Table X it is seen in the former case where two heads are formed as a result of a longitudinal splitting of the polyp, that there is an average increase of 3.4 tentacles per hydra over the number originally possessed by the polyp, while the average gain is but 0.7 tentacles for each hydra when the cut edges reunite to form but one head. The difference between the two is 2.7. The wounds healed in about the same length of time in both experiments and it does not seem as if there could be a much greater amount of new tissue formed in the one case than in the other, so that the cause of the marked difference in the number of tentacles regenerated in the two cases is very difficult to determine. Rand has stated that »the increase of the oral tentacles to an abnormally large number may be regarded as an accidental effect of the stimulus which must always be present at this period«. As injuring the hypostome by a removal of all of the tentacles does not furnish a sufficient stimulus for the production of more tentacles than were removed it is hard to see how this explanation can apply in the case of any other injury to the polyp. It certainly will not account for the marked increase in the number of tentacles in the experiments recorded in Table VIII over those in Table X, as the operation was the same in both sets of experiments.

The number of tentacles normally possessed by Hydra viridis generally varies from six to nine. Individuals with ten tentacles are rarely found and in all the hundreds of polyps examined in the course of these experiments I have only found one with as many as eleven tentacles. In the last two sets of experiments (Tables IX, X) there were found five individuals with eleven tentacles, five with twelve tentacles, three with thirteen tentacles, and two with fourteen tentacles. As this increase in the number of tentacles has been brought about by a reunion of the cut edges after the longitudinal splitting of the body the question suggests itself, whether it will be possible to increase the number of tentacles still further by this means.

The two fourteen-tentacled hydras were first operated upon by dividing the head longitudinally so that each part had seven tentacles. Two heads formed at once, although no attempt was made to keep the cut edges separated. The same operation was repeated on the three thirteen-tentacled hydras, and in two cases double-headed forms were produced as before. In the other polyp the cut edges reunited, but no new tentacles appeared. These results show that there is a limit to the growth of the hypostome. When this is reached a longitudinal cutting of the oral end either causes two heads to form at once or else the cut edges reunite without any further increase in the size of the hypostome or in the number of tentacles.

As observed by RAND, PARKE and others, when a hydra has regenerated more than nine tentacles as a result of such an operation, some of them usually disappear in the course of a few days, either by absorption or by a fusion of two adjacent tentacles, until the number is again brought within the limits of individual normal variation. I have never seen a decrease in the number of tentacles of an originally nine-tentacled hydra as long as the polyp remained in good condition.

c. Double-footed Hydras.

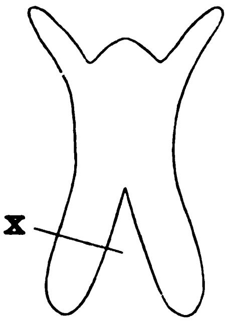
TREMBLEY's experiment of splitting a hydra longitudinally through the aboral end was repeated by MARSHALL who states: »Wenn wir umgekehrt von hinten her den Polyp halb spalten, so vereinigen sich entweder die Spaltungsränder beider Stücke wieder, oder sie schließen sich ebenfalls zu Röhren, aber niemals entwickeln sie am Ende Tentakeln oder eine Mundöffnung. Nach einiger Zeit löst sich vielmehr das eine Stück los, indem es sich langsam abschnürt, so dass, wenn es frei geworden ist, sein vorderer Pol geschlossen erscheint. Dann setzt es sich mit dem hinteren Ende fest und verhält sich nun ganz wie ein durch einen Querschnitt erzieltes Polypenstück, es treibt Tentakeln und bekommt einen Mund.«

For a comparison with the results obtained by splitting a polyp through the oral end, 30 hydras were also split longitudinally through the aboral end, the cut extending in some cases only a short distance through the body, in other cases it reached very near to the circle of tentacles. In 13 of the polyps the cut edges reunited forming normal hydras, although the same means were used to keep the cut edges apart as in the case of the double-headed hydras. The 17 individuals in which the cut edge did not reunite, attached themselves by both aboral ends, each having apparently developed a normal foot in the course of a few hours. My own experiments confirm those of MARSHALL in every respect. The time required for one part to constrict off from the other depends entirely on the extent of the longitudinal cut in the beginning of the experiment, being but a few days in some cases, three or four months in others.

When the two parts come to separate, the line of division never extends through the middle of the polyp as it seems to do when the double-headed forms separate, but the constriction always occurs where the one part is attached to the other. The part which has constricted off (invariably the smaller if the longitudinal cut did not extend exactly through the middle of the polyp) has at first no hypostome and no tentacles. These develop, however, in the course of a few days and a perfectly normal hydra is formed.

Gravity certainly cannot be a factor in causing the separation of the parts when the longitudinal cut is made through the posterior end of a polyp. The process of constricting off of the smaller piece is apparently very much like that which takes place when a bud is pinched off from a parent hydra, only it takes much longer in the former case than in the latter for complete separation to be effected.

Fig. 6.



In varying this experiment, a longitudinal cut was made through the aboral end of the polyp as before. On the following day, one of the aboral ends was removed by a transverse cut as in Fig. 6 *X*, in the hope that a head might develop on the exposed aboral surface. If the cut removed only a small piece of the foot, a new foot invariably formed in the course of a few hours, if the cut was made so that but a small piece remained attached to the body of the polyp it did not develop a foot but became a rounded protuberance on the side of the body of the hydra and was gradually resorbed until a perfectly normal individual was formed. A large piece attached to the body of a hydra thus seems able to exert its individuality sufficiently to regenerate the same structure which had been removed, a small piece on the contrary, loses its individuality entirely and becomes merged into the larger piece.

In twenty other experiments, the head and foot of each polyp were removed and the body then split longitudinally for a short distance through the aboral end. In all cases a head regenerated on the oral end and a foot on each of the exposed aboral surfaces. It was not found possible to produce heteromorphosis by this means.

IV. Grafting Experiments to Produce Heteromorphosis.

The replacement of a part of the body by a part of different kind has been observed by various investigators in many widely different groups of animals. LOEB (6), DRIESCH (2), MORGAN (11), BICKFORD (1) and PEEBLES (14) have observed many cases of heteromorphosis in various species of Tubularia; MORGAN (10) and VAN DUYNE (18) have met with the same phenomenon in Planarians; HERBST (5) has found that antennae like organs often develop when the eyes of certain shrimps are removed; HAZEN (4) has described the regeneration of a head instead of a tail in Allolobophora, and MORGAN (9) has observed the regeneration of a tail in place of a head in the same form; WETZEL (19, 20) and PEEBLES have obtained heteromorphosis in hydra as the result of various experiments in grafting.

In all the following experiments individuals of decidedly different shades of green were united. It was very easy, therefore, to tell the part each kind of tissue played in the subsequent regeneration of new structures, as the line of union of the components of the graft remained visible for several days. In the illustrations given the darker green tissue is dotted in all cases to distinguish it from the lighter colored tissue. The arrows at the side of the figures point in every case towards the anterior end of the hydra.

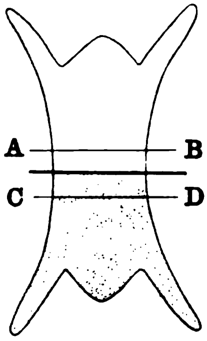
WETZEL'S experiments gave some very interesting results showing the way that regulation to a normal form is brought about in various grafts, and he was able to produce one undoubted case of heteromorphosis. In this graft the foot end of two Hydra grisea had been cut off and the two aboral surfaces united. After the union was complete the head of one component was cut off just back of the circle of tentacles. Five days after the operation two small hook-like processes formed on the exposed oral surface. These hooks later became changed into a foot by which the hydra was attached. When the polyp was sectioned some days later, characteristic gland cells were found in the foot region showing conclusively that a typical foot had been produced on the cut oral surface of the hydra.

PEEBLES repeated the experiment on Hydra grisea and found in but one of fifteen cases that hook-like processes appeared on the oral surface. These, however, never developed into a true foot. In another series of experiments, PEEBLES united the oral surfaces of two polyps after the removal of the tentacles and hypostome. After the two pieces were firmly united, a transverse cut was made through one of the polyps close to the line of union. In five out of twenty-two cases a head developed on the exposed aboral surface of the smaller component of the graft. In the other cases a new head, if it formed at all, was developed at the line of union of the two parts of the graft.

As far as I know these experiments of Wetzel and Peebles give the only unquestionable cases of heteromorphosis in hydra that have been recorded.

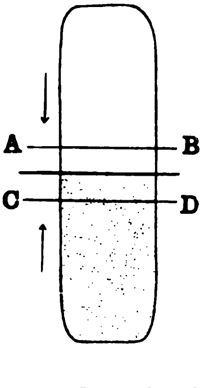
On Jan. 24th, the foot was removed from 20 polyps and the aboral surfaces of the hydra united. The following day one component was cut off very close to the line of union of the two parts as in Fig. 7 *AB*. In nine cases a head developed on the exposed oral surface in the course of the next few days and the two polyps later pulled apart and each formed a normal hydra. In the remaining case, a foot developed on the cut oral surface by which the polyp was attached to the bottom of the dish. No hook-like processes were formed previous to the devolopment of the foot as had been observed in the similar experiments made by Wetzel and Peebles. The polyp resulting from this experiment appeared to be perfectly normal and lived for several weeks without further change.

Fig. 7.



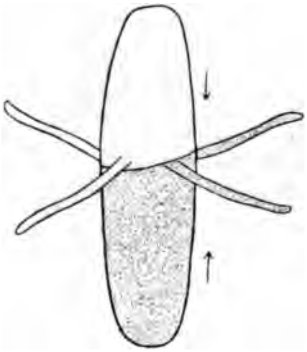
Peebles united two individuals by their oral ends after removing the circle of tentacles, and then cut off both components of the graft to see if the development of the new heads would be modified by the presence of two exposed surfaces. It is not stated whether the cuts were made near the line of union or not. In all the 13 experiments made, a new head grew out in the line of union of the two components.

Fig. 8.



On Jan. 28th, the foot was removed from a light and a dark green hydra and the two aboral ends united. On the following day each component was cut off close to the line of union leaving both pieces as nearly as possible of the same size (Fig. 7 *AB*, *CD*). The operation resulted in the formation of a small ring of tissue with two exposed oral surfaces. Both components of the ring being of the same size neither can be supposed, on account of its greater volume, to exert an influence on the other part. Will each component maintain its individuality and complete itself or will the two pieces merge their individuality and form one perfect polyp? The experiment was made eight times. In every case a normal hydra was formed from the graft, a head developing on one oral surface, a foot on the other. Sometimes the head regenerated on the light colored tissue, sometimes on the dark, but in every case the hypostome and all the tentacles were the color of the tissue on which they were formed. In the course of a few days, however, the line of union of the two components of the graft disappeared and the whole polyp became a uniform shade of green. In five other cases where the transverse cuts had been made about half way between the line of union and the circle of tentacles, a head developed on each of the exposed oral surfaces and the two parts pulled away from each other in about ten days.

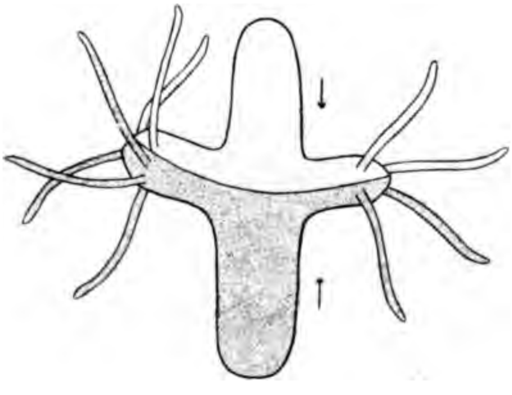
Fig. 9.



In the reverse of this experiment, the heads of two polyps were removed and the oral ends grafted together, after which each part of the graft was cut off close to the line of union leaving pieces as near the same size as possible (Fig. 8 *AB*, *CD*). In each of the eight experiments a normal hydra developed in the course of a week, a head forming on one aboral surface, a foot on the other.

In ten other experiments the parts were grafted as before, but the transverse cuts were made some distance from the line of union. In all these cases tentacles formed at the line of union and sooner or later the two components of the graft separated and became normal hydra. The behavior of the graft was very unusual in one case and this polyp is, perhaps, worthy of a more detailed description. The graft was made on Jan. 29th. On Feb. 1st, four tentacles appeared at about equal distances around the middle of the graft, two of them were light green, the other two much darker (Fig. 9).

Fig. 10.



Five days later a head developed at each side of the middle line, one with six, the other with four tentacles. The line of fusion of the two parts of the graft was still distinct and each head was seen to be formed partly of dark, partly of light colored tissue, the tentacles in each case being the color of the tissue on which they were formed (Fig. 10). During the following week the heads increased in length, seemingly at the expense of both of the components of the graft, as the latter decreased steadily in size during this period (Fig. 11). The line of fusion of the two parts of the graft was no longer visible on Feb. 8th, the whole polyp being a uniform shade of green. On Feb. 12th, the hydra appeared as in Fig. 12. Four days later the two parts separated in the middle line, one hydra

Fig. 11.

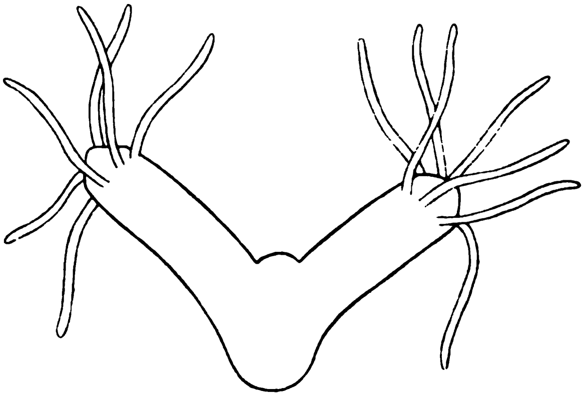
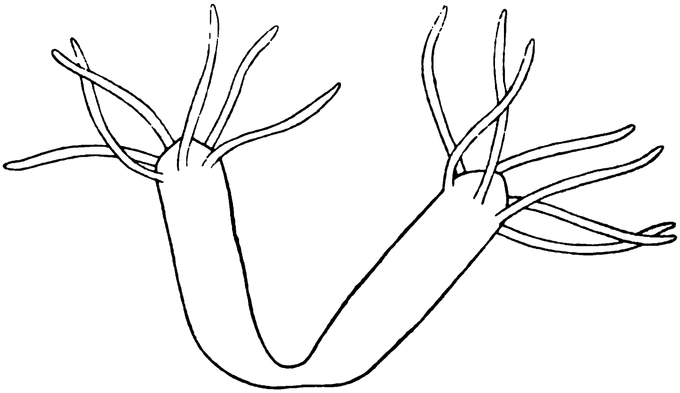


Fig. 12.



having six, the other seven tentacles. Each of the two individuals formed received some tissue from both components of the graft, instead of being produced by a rearrangement of the tissue of one component only as was the case in the other polyps formed in this set of experiments. The polarity of the pieces was not reversed in the formation of the two individuals as the oral end of each piece regenerated into the head of an individual and the aboral end became the foot. After separation, the two polyps lived for nearly two months showing that normal individuals had been formed by the merging of the tissues from two individuals into one.

From the results of the above experiments and of those of the investigators cited, it seems evident that when two polyps are grafted either by the oral or the aboral ends and one or both component subsequently cut off, that in the great majority of cases the parts of the graft maintain their individuality and sooner or later separate from each other and become perfect polyps. If one component of the graft is much larger than the other, it may, in exceptional cases, so influence the smaller piece that the latter loses its own individuality and becomes merged into the larger component the two forming one typical hydra. When both parts of the graft are very small and of nearly the same size, neither of itself becomes a perfect hydra, but the two pieces together form a complete polyp by a permanent fusion of their tissues. The size of the components of the graft is thus apparently an important factor in determining the end result of the experiment, yet it cannot be the only one as is plainly shown by the last set of experiments described. All of the ten experiments in the series were made as nearly as possible in the same way, yet regulation to a normal form was brought about in nine cases by the complete separation of the two parts of the graft, each becoming a typical hydra. In the one exceptional case, two polyps were eventually produced, but each was formed of tissue from both components of the graft. Why in the one graft the union of the two parts should be much more complete than in the others of the series is not at all clear. It is evident that some other factor besides the size of the components of the graft must be at work in the regeneration.

V. Other Experiments to Produce Heteromorphosis.

In experimenting on a tubularian hydroid, Cerianthus membranaceus, Loeb found that if a small triangular piece is cut from the side of the polyp as in Fig. 13 *ABE*. that a new head forms at the cut surface *BE*. In general, the growth of the new head takes place more rapidly and more tentacles are formed the nearer the cutting to the oral pole. If, however, the cut is quite near the aboral pole the formation of a new head does not take place. Thinking it might be possible to obtain heteromorphosis in hydra by the same means a triangular piece was cut from the side of 20 hydras (Fig. 13 *ABE*). Owing to the contractions of the polyps, it was not found possible to remove a similar section in all the experiments, so that pieces of various sizes and from different parts of the body were removed. The result was the same in all cases however, the wound healed quickly without the formation of any new structure at the place of injury.

Fig. 13.

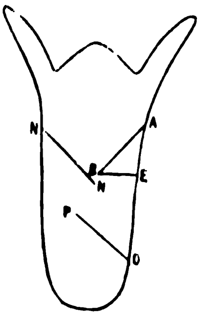
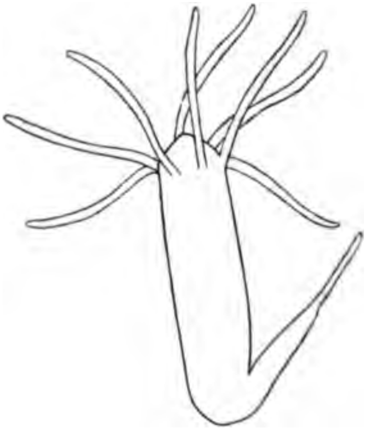


Fig. 14.



In a modification of this experiment an oblique cut was made in 20 polyps as shown in Fig. 13 *NN*, without the removal of any tissue whatever. The cut edges were kept separated for some two hours after the operation in the hope that their reunion might be prevented and a new structure develop at the place of injury. The position of the cut was varied in different cases, sometimes it was made quite close to the circle of tentacles, sometimes it was made near the aboral pole. On the day following the operation the cut-edges had united in 10 individuals and no signs of the injury could be detected. In the other ten polyps a small lateral piece projected from the body of the hydra. The three smallest of these pieces never produced any tentacles but were gradually resorbed into the body of the hydra and a perfectly normal form was again produced in the course of ten days. In the seven remaining cases, a hypostome and several tentacles formed on the smaller part. These heads never have as many tentacles as the original polyps as is shown by the following Table. This result was, of course, to be expected as the hypostome of the new head was always much smaller than that of the old.

Table XI.

No. individuals	No. tentacles on old head	No. tentacles on new head
1	10	8
1	8	7
2	8	6
1	7	6
1	7	5
1*	7	1
Total 7	55	39

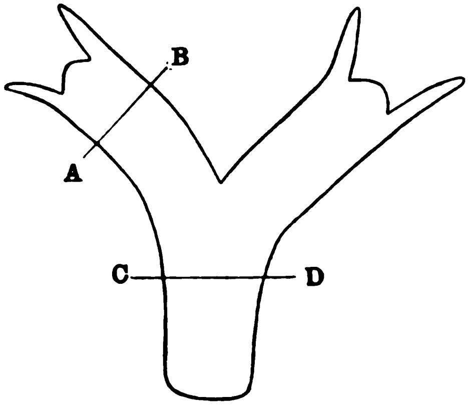
The last case * was rather an interesting one. The one tentacle produced by the lateral piece appeared three days after the operation. No hypostome developed and no more tentacles were ever formed, the one tentacle being a prolongation of the end of the lateral piece (Fig. 14). In the course of a week the tentacle dwindled considerably in size and finally disappeared entirely. Meanwhile the lateral piece became much smaller and was finally resorbed into the larger part and a normal hydra again produced.

In his grafting experiments, Rand found one case in which a graft developed a foot and was later resorbed. He states »if the graft bears tentacles when grafted, or if it regenerates tentacles after the operation, it is destined to separate from the stock. If a piece without tentacles is grafted and if it does not regenerate tentacles, it is destined to be resorbed. It appears, then, that the fate of the graft depends upon its degree of specialization. An indifferent piece bearing none of the oral or aboral organs of a normal polyp is completely resorbed by the stock. A graft bearing a hypostome and tentacles maintains its individuality and eventually becomes a free normal polyp.«

The above experiment which, perhaps, is not strictly comparable to the graft experiment made by Rand since there is probably a stronger union between the parts of the same individual than between the tissues of two different polyps, shows that, in exceptional cases, a piece can be resorbed even after it has become so specialized as to produce a tentacle. Why resorption takes place in this case and not in the other cases in the same set of experiments is not at all clear as the lateral piece in this case was seemingly as large as the other ones which produced typical heads with several tentacles.

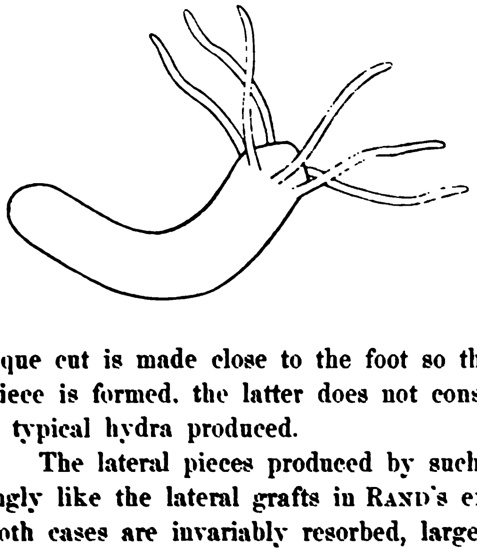
When heads were formed on the lateral pieces, they invariably separated from the body of the hydra in the course of two or three weeks and became complete individuals. During this time the connection between the two gradually decreased until, for two or three days before the final separation, the two polyps were attached only by a very slender prolongation of the foot which later broke and the two hydras were set free.

Fig. 15.



When an oblique cut is made from near the aboral pole of a polyp towards the oral end (Fig. 13 *PO*), the larger piece containing the head regenerates a foot on the cut aboral surface in the course of a few days and in two or three weeks, if the smaller piece can maintain its individuality and constrict off from the larger part, the two pieces separate, each forming a normal hydra. If the oblique cut is made close to the foot so that only a very small lateral piece is formed, the latter does not constrict off, but is resorbed and a typical hydra produced.

Fig. 16.



The lateral pieces produced by such oblique cuts behave strikingly like the lateral grafts in Rand's experiments. Small pieces in both cases are invariably resorbed, larger ones regenerate a head or a foot on the free end, depending on which had been removed, and later the two pieces separate from each other and become complete individuals. There is, however, one difference between the lateral grafts and the lateral pieces produced by oblique cuts. In the former case the graft always migrates to the foot of the stock whether it is to be resorbed or to be separated from the stock, while in the latter case small pieces are resorbed in situ, only the larger ones appear to migrate towards the foot of the hydra before separation is effected.

In investigating the regeneration of hydra split longitudinally through the oral end, one of the heads was cut off of 26 double-headed polyps as shown in Fig. 15 *AB*. The position of the cut varied somewhat in different cases. Sometimes it was close behind the circle of tentacles, sometimes it was near the junction of the two heads. Will a head or a foot develop on the exposed oral surface *AB*? When but a small piece remained attached to the body of the polyp a new head never developed on the cut surface, but the piece appeared for some time as a knob-like protuberance on the side of the polyp and was later resorbed. In all but one of the 18 cases in which the cut had been made close behind the circle of tentacles, a new head developed on the cut surface in the course of three days. In the one exceptional case, the wounded surface healed over but no hypostome or tentacles had developed ten days after the operation. The body of the hydra was then cut off as in Fig. 15 *CD*. The day following the operation, the cut edges had united and the polyp appeared as in Fig. 16. Four days later the hydra had straightened out to the normal form and was attached by a foot which had developed at the cut surface *AB*. The hydra lived for two months without undergoing any further change. The same experiment was repeated later 25 times, but no other case of heteromorphosis was obtained, the larger pieces invariably regenerated a new head, the smaller ones were resorbed.

VI. The Grafting of a Number of Pieces of Hydra Oriented in the Same and Opposite Directions.

PEEBLES made two grafting experiments in which pieces of three different hydras were united as shown in Fig. 17. Two of the pieces were oriented in the same direction, the third was reversed so that an oral surface was exposed at each end of the graft. The history of these polyps is as follows: »A few days after the experiment the polyp was attached at two points, at the foot end of *C*, and the

Fig. 17.

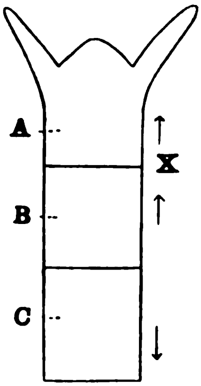


Fig. 18.

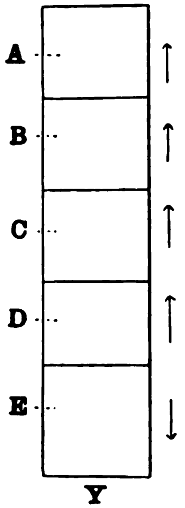
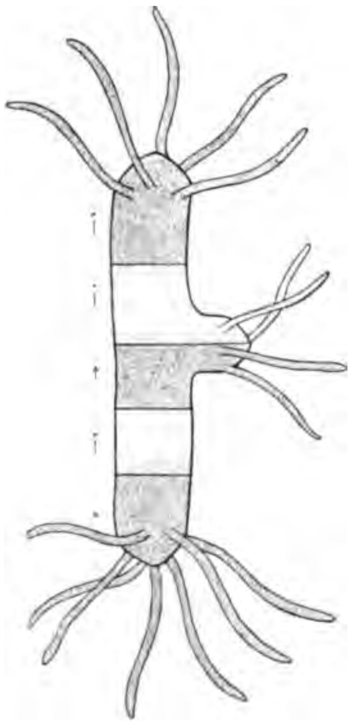


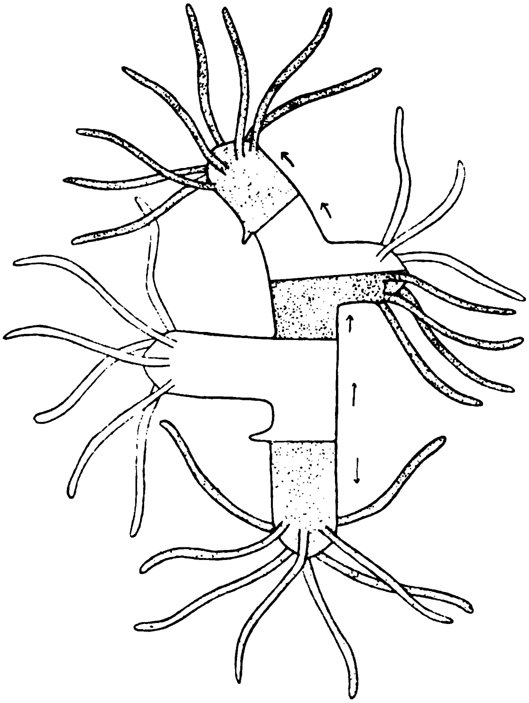
Fig. 19.



combined aboral surfaces of *A* and *B*, the bodies of *B* and *C* forming an arch. In one graft the foot at *X* soon freed itself and the polyp took the normal position indicated in Fig. 17. In the other the component *A* tore apart from *B* and *C*, a new head formed at the line of union of the oral surfaces of *B* and *C*, and these two components merged into one. The fate of these polyps was not determined as they died in the course of three or four weeks.

On Jan. 22, the head and foot of five hydras were cut off and the bodies were grafted together as shown in Fig. 18. The pieces were all oriented in the same direction except component *E* which was reversed so that an oral surface was exposed at both ends of the graft as in the experiments made by Peebles. Will the posterior component of the graft be able to maintain its individuality and regenerate a head on the exposed oral surface at *Y* or will its polarity be reversed and a foot develop making one individual of normal shape out of the five components of the graft? As found to be the case in many of the grafting experiments made by Wetzel and Peebles, the pieces did not unite to form a single individual. On Jan. 26th, the graft had developed three heads (Fig. 19). One at each of the exposed oral surfaces at the ends of the graft, and a third at the line of union of *B* and *C*. The last head was formed in part of the oral surface of *C* and in part of the aboral surface of *B* and is, therefore, a partial heteromorphic production. On Jan. 30th, a fourth head developed at the anterior end of component *D* and two hook-like processes appeared, one at the posterior end of *A*, the other at the aboral end of *D*, by which the graft was attached (Fig. 20). Meanwhile, although the polyp seemed to be in good condition, it continually decreased it size. During the following week, the heads grew considerably in length and the number of tentacles was increased so that the two end heads each had eight tentacles, the third had seven and the last head to appear had six tentacles (Fig. 20). There was no sign of a constriction in any part of the body or of the development of a foot during the next few days. On Feb. 19th, the two heads in the middle of the graft had shifted their position so that they were directly opposite each other and when the polyp was expanded it appeared as a cross with a head at the end of each arm. On Feb. 23rd, the polyp seemed much contracted and two days later it died.

Fig. 20.



On Jan. 25th, five pieces of hydra were grafted together after the removal of the head and foot of each polyp, all the pieces being oriented in the same direction. On Jan. 30th, a head with five tentacles had developed at one end but no foot had developed at the aboral surface of the fifth component of the graft. On Feb. 6th, a second head had developed at the anterior end of the fourth component (Fig. 21) and two small hook-like processes had formed at the aboral ends of the second and fourth component, the fifth piece of the graft had not regenerated in any way. During the next two weeks the whole polyp shortened considerably and the two heads were much larger although no more tentacles had regenerated. A bud developed at the posterior end of the first component and was constricted off on Feb. 12th. On Feb. 23rd, another bud was given off from the same place. Meanwhile, the fifth component had decreased considerably in size without regenerating in any way and it was

Fig. 21.

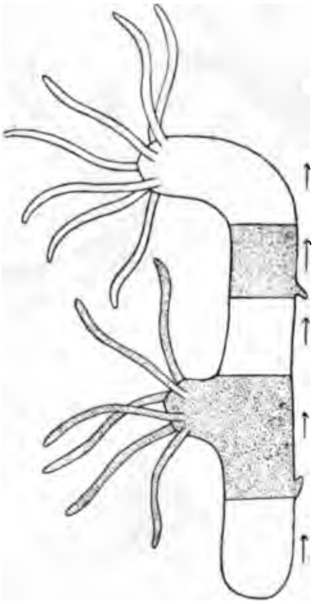
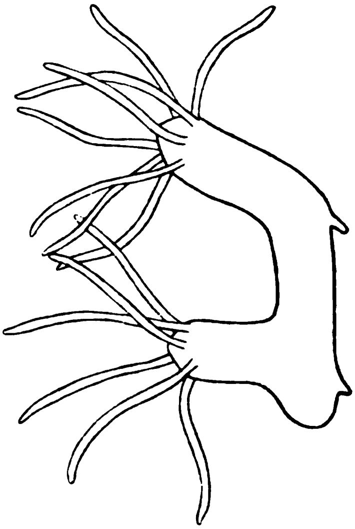


Fig. 22.



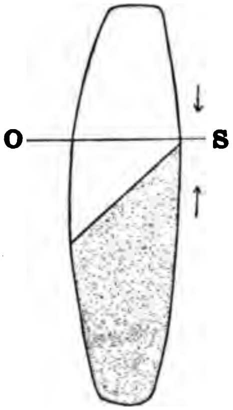
apparently being resorbed into the hydra head near it as the latter constantly increased in length (Fig. 22). On Feb. 26th, the fifth component had entirely disappeared and the two hydra heads were connected only by a narrow strip of substance on which were the two hook-like processes by which the polyp was attached. On March 5th, the connection between the two individuals was much like that shown in Fig. 12. On March 12th, the two polyps separated forming two normal individuals as each of the small hook-like processes by which the polyp had been attached changed into a typical foot. The two polyps were not abnormally large when they separated, one had eight, the other seven tentacles. They were watched for three weeks after the final separation and no further change noticed in them.

These two experiments show that several pieces of hydra can be grafted together so that they will remain attached for many weeks although a permanent union of all of the parts is not possible. One individual cannot be formed by this means, yet each component of the graft does not maintain its own individuality sufficiently to produce a normal polyp. As many individuals are finally produced from the graft as there are heads formed. The new individuals being formed of parts of two, perhaps of three different polyps in the second experiment. The tissues have fused completely in these cases so that a permanent union is effected and perfectly normal hydras formed.

VII. Oblique Grafts.

In all the experiments made by Wetzel and Peebles and in all the preceding ones described, transversely cut surfaces were united. Is it possible to form grafts when the surfaces are cut obliquely and if so what is the fate of such grafts?

Fig. 23.



These experiments are somewhat difficult to make as the edges of the cut show a very great tendency to curl in at once and it is almost impossible to get the two surfaces in good contact. On Jan. 18th, the head of a light and of a dark green polyp was cut off by an oblique cut and the two oral surfaces united as in Fig. 23. The following day one piece was cut off transversely quite close to the line of union (Fig. 23 *OS*). Will the individuality of the larger piece predominate as in the case of the experiments made by Wetzel and Peebles and a head form on the cut aboral surface? Twenty experiments were made, but in only two cases were the edges perfectly united so that a straight tube was formed. In one of these polyps the exposed aboral surface healed over and no new structure was developed until Jan. 26th, when two heads appeared as shown in Fig. 24. One hypostome and all of its tentacles was composed entirely of the light colored tissue of the smaller piece, while the other head was made up of tissue from both components of the graft although the light colored tissue predominated and but two of the six tentacles were dark. Whether this can be called a case of heteromorphosis is doubtful. The polarity of the light colored component was not reversed as the new heads regenerated from the oral end of the smaller component and the fact that two heads formed instead of one shows that the smaller piece did not lose its individuality but was able to influence the regeneration to a considerable extent. The two heads gradually increased in size, the connection between them decreased and on Feb. 21st, final separation was effected and two normal polyps formed.

Fig. 24.

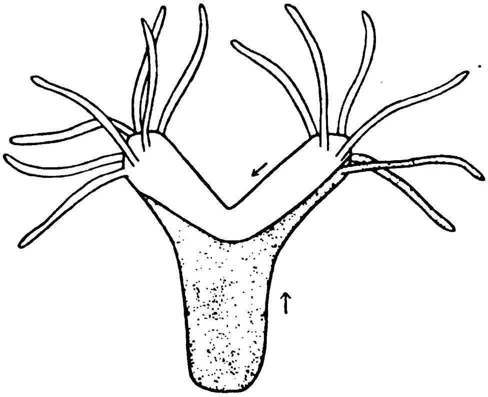


Fig. 25.

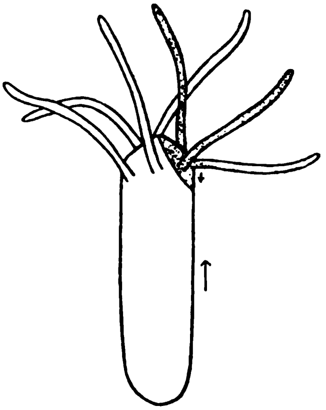


Fig. 26.

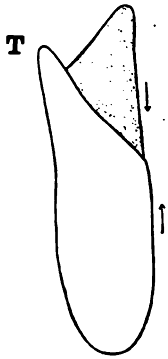
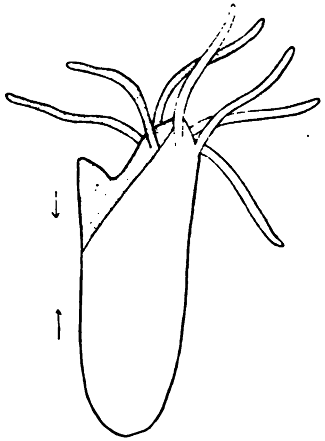


Fig. 27.



In the other case in which perfect union of the two pieces had been effected, a single head formed on the cut surface, but it was composed of tissue from both components (Fig. 25). The smaller component lost its individuality and behaved almost as indifferent tissue capable of developing into any structure needed to complete the individual, as its polarity was reversed and its substance worked over into the new head. The polyp thus formed was kept under observation for two months and showed no further change.

In the other experiments in this group, the cut edges of the two pieces of hydra did not fuse completely. On the day following the operation the graft appeared as in Fig. 26. The smaller component was then cut off transversely so that an oral and an aboral surface were exposed at the same end of the graft. The result was the same in all cases. A head developed on the oral end of the larger component (Fig. 26 *T*). The smaller piece was resorbed without regenerating a new structure and a normal polyp was produced. In one case only (Fig. 27) the smaller component took part in the formation of the new head on the oral surface as half of the hypostome and three of the tentacles were the color of the smaller piece, the rest were like the larger component.

Fig. 28.

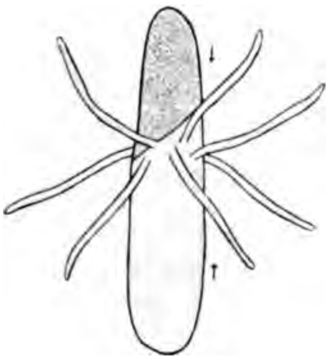
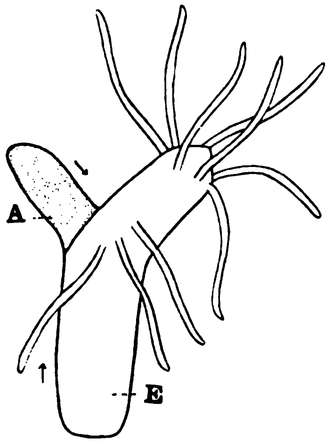


Fig. 29.



In another set of experiments made on Jan. 22nd, two pieces of hydra were united by their oral ends as before, but each component of the graft was cut off transversely near the foot making the two parts as nearly as possible of the same size in order to see whether the two components would so influence each other that a normal individual would be produced or whether each part would maintain its own individuality and form a complete polyp. In eight of the 10 experiments in this set a head appeared on one component of the graft at the line of union. Later the other component constricted off and developed a hypostome and several tentacles so that a normal polyp was formed from each part of the graft.

In another individual of the set two heads budded out, one on each side of the line of union of the two components. Each head was formed partly of light, partly of dark colored tissue as in the case of the transverse graft shown in Fig. 10. In the course of three weeks the two heads separated and two normal polyps were formed.

In the remaining case a number of tentacles appeared at the line of union three days after the operation (Fig. 28). On Jan. 30th, a hypostome developed in this region. The new head pushed out at one side leaving three tentacles below it on the body of the hydra (Fig. 29). As no new structure had developed on *A* by Feb. 4th, the distal end was cut off in the hope that a head might regenerate on the exposed aboral surface. The piece remaining attached to *E* did not regenerate at all but decreased in size and was finally resorbed as were also the superfluous tentacles in the middle of the body. A normal individual was thus formed from the graft.

Fig. 30.

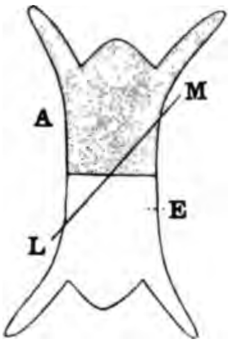
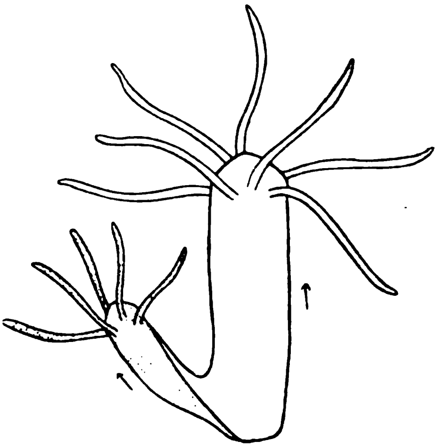


Fig. 31.



In a third set of experiments, the aboral end of two polyps was cut off by a transverse cut and the two aboral surfaces united. After union was complete, an oblique cut was made through both components (Fig. 30 *LM*) so that each piece had at its cut end part of an oral and part of an aboral surface.

In three cases but a very small piece was left attached to the larger component and in these cases the smaller pieces were resorbed and a foot developed on the aboral end of the other piece.

In one instance the oblique cut had been made so that a somewhat larger piece remained attached to component *E*. A head with five tentacles developed entirely from the darker green tissue of the small piece (Fig. 31) and later this head pinched off and became a separate individual.

The regeneration of these oblique grafts is very similar to that of transverse grafts. It both components of the graft are of sufficient size, each maintains its own individuality and forms a complete individual. If one part is much smaller than the other it is usually resorbed into the body of the larger part and a normal polyp produced. In certain experiments, both components took part in the formation of a new structure the union of the tissues in this case being a permanent one. When a head is formed of two kinds of tissue each tissue regenerates tentacles like itself and is not influenced by the other component of the graft.

Summary.

1) New polyps formed from the body of a Hydra viridis usually regenerate fewer tentacles than were removed, and the greater the original number of tentacles the greater the number of tentacles regenerated. Rand obtained the same result in a similar set of experiments.

2) When the head of a hydra is cut off and a small individual formed from the anterior piece, there is no decrease in the number of tentacles to correspond with the smaller size of the new polyp.

3) There is considerable individual variation in the size of the hypostomes of normal polyps bearing the same number of tentacles; but in general, the size of the hypostome is directly proportional to the number of tentacles.

4) In polyps remodelled from the posterior part of Hydra viridis the size of the hypostome is less than that of the original hydra and is directly proportional to the number of tentacles born by the new individual.

5) In a polyp remodelled from the anterior piece of a Hydra viridis, the hypostome decreases in size to correspond with the size of the new individual although there is no decrease in the number of tentacles.

6 When all the tentacles of a polyp are cut off close to the hypostome the cutting does not give a stimulus to the production of an increased number of tentacles, as the same or a smaller number of tentacles always regenerates after such an operation.

7 Double-headed hydras can be produced by a longitudinal splitting of the oral end of a polyp. The total number of tentacles on the new heads is always greater than that of the original number of tentacles. The average increase being 5.1 tentacles per hydra.

8 If the head of a polyp is cut off and the body then split longitudinally through the oral end, the total number of tentacles on the double-headed forms produced is greater than the original number of tentacles by an average of 3.4 tentacles per hydra.

9 The two polyps formed by the longitudinal splitting of the oral end of a hydra separate entirely after a time and each becomes a normal individual. The amount of time required for separation depends entirely upon the extent of the original cut. Gravity is not the cause of this phenomenon as separation is effected just as readily when the double-headed polyp is fixed in an inverted position.

10 When the cut edges unite, after a longitudinal splitting of the oral end of a polyp, there is always an increase in the number of tentacles whether the head is removed before the longitudinal cut is made or not. This increase is probably due to the fact that a rapid growth of new tissue takes place at the region of injury so that the hypostome is increased in size.

11 Double-footed hydras can be produced by a longitudinal splitting of the aboral end of a polyp. Regulation to a normal form is brought about in such cases by the pinching off of one part of the polyp and the development of a head on its free oral end. This result agrees with that obtained by Marshall in similar experiments.

12 When two individuals are united by their aboral surfaces after the removal of the foot ends, and one component of the graft subsequently cut off close to the line of union, a head is usually formed on the exposed oral surface, although in exceptional cases a foot developes so that a normal polyp is produced.

13 When two polyps are grafted together by their aboral surfaces, after the removal of the foot ends, and each component of the graft subsequently cut off close to the line of union so that both components are practically of the same size, a head forms on one oral surface and a foot on the other so that a normal individual is produced. If the transverse cuts are made some distance from the

line of union, a head developes on each of the exposed oral surfaces and the two components of the graft pull apart and each becomes a typical hydra.

14) If the heads of two polyps are cut off and the exposed oral ends grafted together, each component of the graft being then cut off close to the line of union so that the two pieces are nearly of the same size, normal polyps are produced by the regeneration of a head on one aboral surface and the regeneration of a foot on the other. When the transverse cuts are made some distance from the line of union, tentacles always appear at the middle of the graft and later two complete individuals are formed.

15) If a triangular piece is cut from the side of a hydra, the wound heals without the formation of any new structure at the place of injury.

16) When an oblique cut is made from near the head of a polyp to the foot region, small lateral pieces are produced when the cut edges do not reunite. If these lateral pieces are small they are resorbed and normal polyps again formed, if the lateral pieces are large they develop a head which never has as many tentacles as the original hydra, and later the two polyps separate as complete individuals.

17) When an oblique cut is made from near the aboral pole towards the head the result is, in general, the same as in the preceding case. Small pieces are resorbed, larger ones regenerate a foot and later constrict off as separate individuals.

18) One individual is not formed from the parts of five hydras grafted together, neither can each component of such a graft maintain its individuality and form a complete polyp. Eventually as many individuals are formed from the graft as there are heads produced.

19) When, in the new individual, both components of the graft take part in the formation of the new head, each kind of tissue forms tentacles like itself in color and is not influenced by the other component of the graft in this respect at least.

20) When pieces of hydra are grafted together by obliquely cut oral or aboral surfaces and then one or both components cut across transversely, the result is very similar to that of transverse grafts. If both components of the graft are made the same size, each maintains its own individuality and forms a complete individual. If one part is smaller than the other the smaller one is usually resorbed and a normal hydra produced. In some cases both components of

6) When all the tentacles of a polyp are cut off close to the hypostome the cutting does not give a stimulus to the production of an increased number of tentacles, as the same or a smaller number of tentacles always regenerates after such an operation.

7) Double-headed hydras can be produced by a longitudinal splitting of the oral end of a polyp. The total number of tentacles on the new heads is always greater than that of the original number of tentacles. The average increase being 5.1 tentacles per hydra.

8) If the head of a polyp is cut off and the body then split longitudinally through the oral end, the total number of tentacles on the double-headed forms produced is greater than the original number of tentacles by an average of 3.4 tentacles per hydra.

9) The two polyps formed by the longitudinal splitting of the oral end of a hydra separate entirely after a time and each becomes a normal individual. The amount of time required for separation depends entirely upon the extent of the original cut. Gravity is not the cause of this phenomenon as separation is effected just as readily when the double-headed polyp is fixed in an inverted position.

10) When the cut edges unite, after a longitudinal splitting of the oral end of a polyp, there is always an increase in the number of tentacles whether the head is removed before the longitudinal cut is made or not. This increase is probably due to the fact that a rapid growth of new tissue takes place at the region of injury so that the hypostome is increased in size.

11) Double-footed hydras can be produced by a longitudinal splitting of the aboral end of a polyp. Regulation to a normal form is brought about in such cases by the pinching off of one part of the polyp and the development of a head on its free oral end. This result agrees with that obtained by MARSHALL in similar experiments.

12) When two individuals are united by their aboral surfaces after the removal of the foot ends, and one component of the graft subsequently cut off close to the line of union, a head is usually formed on the exposed oral surface, although in exceptional cases a foot developes so that a normal polyp is produced.

13) When two polyps are grafted together by their aboral surfaces, after the removal of the foot ends, and each component of the graft subsequently cut off close to the line of union so that both components are practically of the same size, a head forms on one oral surface and a foot on the other so that a normal individual is produced. If the transverse cuts are made some distance from the

line of union, a head developes on each of the exposed oral surfaces and the two components of the graft pull apart and each becomes a typical hydra.

14) If the heads of two polyps are cut off and the exposed oral ends grafted together, each component of the graft being then cut off close to the line of union so that the two pieces are nearly of the same size, normal polyps are produced by the regeneration of a head on one aboral surface and the regeneration of a foot on the other. When the transverse cuts are made some distance from the line of union, tentacles always appear at the middle of the graft and later two complete individuals are formed.

15) If a triangular piece is cut from the side of a hydra, the wound heals without the formation of any new structure at the place of injury.

16) When an oblique cut is made from near the head of a polyp to the foot region, small lateral pieces are produced when the cut edges do not reunite. If these lateral pieces are small they are resorbed and normal polyps again formed, if the lateral pieces are large they develop a head which never has as many tentacles as the original hydra, and later the two polyps separate as complete individuals.

17) When an oblique cut is made from near the aboral pole towards the head the result is, in general, the same as in the preceding case. Small pieces are resorbed, larger ones regenerate a foot and later constrict off as separate individuals.

18) One individual is not formed from the parts of five hydras grafted together, neither can each component of such a graft maintain its individuality and form a complete polyp. Eventually as many individuals are formed from the graft as there are heads produced.

19) When, in the new individual, both components of the graft take part in the formation of the new head, each kind of tissue forms tentacles like itself in color and is not influenced by the other component of the graft in this respect at least.

20) When pieces of hydra are grafted together by obliquely cut oral or aboral surfaces and then one or both components cut across transversely, the result is very similar to that of transverse grafts. If both components of the graft are made the same size, each maintains its own individuality and forms a complete individual. If one part is smaller than the other the smaller one is usually resorbed and a normal hydra produced. In some cases both components of

6) When all the tentacles of a polyp are cut off close to the hypostome the cutting does not give a stimulus to the production of an increased number of tentacles, as the same or a smaller number of tentacles always regenerates after such an operation.

7) Double-headed hydras can be produced by a longitudinal splitting of the oral end of a polyp. The total number of tentacles on the new heads is always greater than that of the original number of tentacles. The average increase being 5.1 tentacles per hydra.

8) If the head of a polyp is cut off and the body then split longitudinally through the oral end, the total number of tentacles on the double-headed forms produced is greater than the original number of tentacles by an average of 3.4 tentacles per hydra.

9) The two polyps formed by the longitudinal splitting of the oral end of a hydra separate entirely after a time and each becomes a normal individual. The amount of time required for separation depends entirely upon the extent of the original cut. Gravity is not the cause of this phenomenon as separation is effected just as readily when the double-headed polyp is fixed in an inverted position.

10) When the cut edges unite, after a longitudinal splitting of the oral end of a polyp, there is always an increase in the number of tentacles whether the head is removed before the longitudinal cut is made or not. This increase is probably due to the fact that a rapid growth of new tissue takes place at the region of injury so that the hypostome is increased in size.

11) Double-footed hydras can be produced by a longitudinal splitting of the aboral end of a polyp. Regulation to a normal form is brought about in such cases by the pinching off of one part of the polyp and the development of a head on its free oral end. This result agrees with that obtained by MARSHALL in similar experiments.

12) When two individuals are united by their aboral surfaces after the removal of the foot ends, and one component of the graft subsequently cut off close to the line of union, a head is usually formed on the exposed oral surface, although in exceptional cases a foot developes so that a normal polyp is produced.

13) When two polyps are grafted together by their aboral surfaces, after the removal of the foot ends, and each component of the graft subsequently cut off close to the line of union so that both components are practically of the same size, a head forms on one oral surface and a foot on the other so that a normal individual is produced. If the transverse cuts are made some distance from the

line of union, a head developes on each of the exposed oral surfaces and the two components of the graft pull apart and each becomes a typical hydra.

14) If the heads of two polyps are cut off and the exposed oral ends grafted together, each component of the graft being then cut off close to the line of union so that the two pieces are nearly of the same size, normal polyps are produced by the regeneration of a head on one aboral surface and the regeneration of a foot on the other. When the transverse cuts are made some distance from the line of union, tentacles always appear at the middle of the graft and later two complete individuals are formed.

15) If a triangular piece is cut from the side of a hydra, the wound heals without the formation of any new structure at the place of injury.

16) When an oblique cut is made from near the head of a polyp to the foot region, small lateral pieces are produced when the cut edges do not reunite. If these lateral pieces are small they are resorbed and normal polyps again formed, if the lateral pieces are large they develop a head which never has as many tentacles as the original hydra, and later the two polyps separate as complete individuals.

17) When an oblique cut is made from near the aboral pole towards the head the result is, in general, the same as in the preceding case. Small pieces are resorbed, larger ones regenerate a foot and later constrict off as separate individuals.

18) One individual is not formed from the parts of five hydras grafted together, neither can each component of such a graft maintain its individuality and form a complete polyp. Eventually as many individuals are formed from the graft as there are heads produced.

19) When, in the new individual, both components of the graft take part in the formation of the new head, each kind of tissue forms tentacles like itself in color and is not influenced by the other component of the graft in this respect at least.

20) When pieces of hydra are grafted together by obliquely cut oral or aboral surfaces and then one or both components cut across transversely, the result is very similar to that of transverse grafts. If both components of the graft are made the same size, each maintains its own individuality and forms a complete individual. If one part is smaller than the other the smaller one is usually resorbed and a normal hydra produced. In some cases both components of

the graft take part in the formation of a new structure, the union of tissues in these cases is a permanent one and each tissue regenerates new tissue like itself.

Bryn Mawr College, Bryn Mawr, Pa., May 6th 1901.

Zusammenfassung.

1 Die vom Körper einer Hydra viridis neugebildeten Polypen bekommen gewöhnlich weniger Tentakel wieder als entfernt wurden, und je größer die ursprüngliche Tentakelzahl war, desto größer ist die Zahl der regenerirten Tentakel. RAND erhielt dasselbe Resultat bei einer ähnlichen Versuchsfolge.

2 Wenn das Kopfende einer Hydra abgeschnitten ist und ein kleines Einzelthier aus dem vorderen Theilstück sich bildet, so bemerkt man keine Abnahme in der Tentakelzahl, etwa im Verhältnis zur geringeren Größe des neuen Polypen.

3) Es bestehen beträchtliche Größenschwankungen der Hypostome bei Polypen mit derselben Tentakelanzahl: im Allgemeinen ist aber die Hypostomgröße direkt proportional der Tentakelanzahl.

4) Bei Polypen aus dem Hintertheil von Hydra viridis, die wieder ihre alte Form gewonnen haben, ist die Hypostomgröße geringer als beim Ausgangspolypen und direkt proportional der Tentakelzahl, die das neue Thier hervorbringt.

5) Bei einem aus einem vorderen Theilstück von Hydra viridis wiedererstandenen Polypen nimmt das Hypostom, mit der Größe des neuen Individuums in Beziehung stehend, an Größe ab, obwohl eine Verminderung der Tentakelzahl nicht stattfindet.

6) Wenn alle Tentakel eines Polypen dicht am Hypostom abgeschnitten werden, giebt der Schnitt keinen Reiz zur Bildung einer größeren Anzahl von Tentakeln ab, da sich dieselbe oder eine geringere Anzahl von Tentakeln nach der Operation regenerirt.

7, Doppelköpfige Hydras können durch Längsspaltung des Mundendes eines Polypen hervorgebracht werden. Die Gesammtzahl der Tentakel auf den neuen Köpfen ist stets größer als die ursprüngliche Zahl der Tentakel. Die durchschnittliche Tentakelvermehrung bei dieser Verdoppelung des Kopfes beträgt 5,1 Tentakel pro Hydra.

8. Schneidet man einem Polypen den Kopf ab und spaltet man seinen Körper der Länge nach durch das Mundende, so ist die Gesammtzahl der Tentakel an den so entstandenen Doppelkopfthieren durchschnittlich um 3,4 Tentakel auf den Polypen größer als die ursprüngliche Zahl derselben.

9) Die zwei durch Längsspaltung des Mundendes einer Hydra erzeugten Polypen trennen sich nach einiger Zeit vollständig und jeder wird zu einem normalen Einzelthier. Die erforderliche Zeitdauer bis zur Trennung hängt gänzlich von der Ausdehnung des ursprünglichen Schnittes ab. Die Schwerkraft ist nicht die Ursache dieser Erscheinung, da die Trennung ebenso gut erfolgt, wenn der zweiköpfige Polyp in umgekehrter Lage fixirt wird.

10) Wenn sich die Schnittränder nach einer Längsspaltung des Kopfendes eines Polypen vereinigen, so entsteht immer eine Vergrößerung der Tentakelzahl, gleichgültig ob der alte Kopf vor der Längsspaltung entfernt wurde oder nicht. Die Vermehrung rührt möglicher Weise von dem Umstande her, dass

ein rapides Gewebswachsthum im Verletzungsgebiet Platz greift, so dass das Hypostom an Größe zunimmt.

11) Hydras mit zwei Füßen können durch Längsspaltung des aboralen Polypenendes erzeugt werden. Die Rückkehr zur Normalform kommt durch Abschnürung eines Theils des Polypen und Entwickelung eines Kopfes an seinem freien Ende zu Stande. Dieses Resultat stimmt mit dem von MARSHALL bei ähnlichen Versuchen erhaltenen.

12) Vereinigt man zwei Individuen mit ihren aboralen Oberflächen nach Entfernung der Fußenden und schneidet man nachträglich den einen Komponenten dicht über der Vereinigungsstelle ab, so bildet sich gewöhnlich ein Kopf an der freien oralen Oberfläche, wenn auch ausnahmsweise sich ein Fuß entwickelt und somit ein normaler Polyp entsteht.

13) Werden zwei Polypen mit ihren aboralen Seiten nach Entfernung der Fußenden zusammengeheilt und nachträglich beide Komponenten dicht am Vereinigungsniveau so abgeschnitten, dass beide so gut wie dieselbe Größe erhalten, so bildet sich ein Kopf an der einen oralen Oberfläche und ein Fuß an der anderen, so dass ein normales Individuum entsteht. Werden die Querschnitte in einiger Entfernung von der Vereinigungsstelle angelegt, so entsteht an jeder der freien Oberflächen ein Kopf, die beiden Komponenten trennen sich und jeder wird zu einer typischen Hydra.

14) Wenn die Köpfe zweier Polypen abgeschnitten und die freien Mundenden vereinigt werden, worauf jeder Komponent unter Innehaltung möglichst gleicher Größe nahe an der Vereinigungsstelle abgeschnitten wird, so entstehen normale Polypen durch die Regeneration eines Kopfes an der einen aboralen Schnittfläche und die eines Fußes an der anderen. Werden die Querschnitte in einiger Entfernung von der Vereinigungsstelle angelegt, so bilden sich stets Tentakel in der Mitte der Narbe und später entstehen zwei vollständige Individuen.

15) Wird ein dreieckiges Stück aus der Seite einer Hydra geschnitten, so heilt die Wunde ohne irgendwelche Neubildung an der Verletzungsstelle.

16) Wird ein Schrägschnitt von nahe am Kopf eines Polypen gegen dessen Fußende hin geführt, so bilden sich kleine seitliche Stücke an, falls die Schnittränder sich nicht vereinigen. Sind diese seitlichen Ansätze nur klein, so werden sie resorbirt und es entstehen wieder normale Polypen, sind sie aber bedeutend, so bilden sie einen Kopf, der aber niemals so viele Tentakel hat, als die ursprüngliche Hydra und später trennen sich die beiden Polypen als vollständige Einzelthiere.

17) Wird ein Schrägschnitt vom aboralen Ende her gegen den Kopf hin geführt, so ist das Resultat im Allgemeinen dasselbe als im vorigen Falle. Kleine Neubildungen werden resorbirt, größere bilden einen Fuß und schnüren sich später als vollständige Individuen ab.

18) Aus den Theilen von fünf mit einander vereinigten Hydras lässt sich nur ein Individuum nicht bilden. Es kann aber auch nicht jeder Komponent einer solchen Vereinigung seine Individualität bewahren und zu einem vollständigen Individuum werden. Eventuell werden so viele Einzelthiere von der Vereinigung gebildet, als sie Köpfe hervorbringt.

19) Wenn in einem neugebildeten Individuum beide Komponenten an der Neubildung des Kopfes betheiligt sind, so bildet jede Gewebsart Tentakel, welche ihr selbst in der Farbe gleichen und ist wenigstens in dieser Beziehung von dem anderen Komponenten nicht beeinflusst.

20) Werden nach der Vereinigung zweier am oralen oder aboralen Ende schräg abgeschnittener Hydras beide oder nur ein Komponent quer abgeschnitten, so ist das Resultat sehr ähnlich dem bei Quervereinigungen. Werden beide Komponenten einander gleich gemacht, so hält jeder seine Individualität aufrecht und bildet ein vollständiges Individuum. Ist die eine Hälfte kleiner als die andere, so wird sie gewöhnlich resorbirt und es entsteht eine normale Hydra. In manchen Fällen betheiligen sich beide Komponenten an einer Neubildung; die Vereinigung der Gewebe ist diesfalls eine dauernde und jedes Gewebe erzeugt neues, welches ihm selbst ähnlich ist.

Literature.

1) Bickford, E., Notes on Regeneration and Heteromorphosis of Tubularian Hydroids. Journ. Morph. Vol. IX. 1894.
2) Driesch, H., Studien über das Regulationsvermögen der Organismen. I. Von den regulativen Wachsthums- und Differenzirungsfähigkeiten der Tubularia. Archiv f. Entwickelungsmech. Bd. V. 1897.
3) —— Studien über das Regulationsvermögen der Organismen. V. Ergänzende Beobachtungen an Tubularia. Archiv f. Entwickelungsmech. Bd. XI. 1901.
4) Hazen, A. P., The Regeneration of a Head instead of a Tail in an Earthworm. Anat. Anz. Bd. XVI. 1899.
5) Herbst, C., Über die Regeneration von antennenähnlichen Organen an Stelle von Augen. Archiv f. Entwickelungsmech. Bd. II. 1896.
6) Loeb, J., Untersuchungen zur physiologischen Morphologie der Thiere. I. Über Heteromorphosis. Würzburg 1891.
7) Marshall, W., Über einige Lebenserscheinungen d. Süßwasserpolypen u. üb. eine neue Form von Hydra viridis. Zeitschr. f. wiss. Zool. Bd. XXXVII. 1882.
8) Morgan, T. H., Experimental Studies of the Regeneration of Planaria maculata. Archiv f. Entwickelungsmech. Bd. VII. 1898.
9) —— A Confirmation of Spallanzani's Discovery of an Earthworm Regenerating a Tail in place of a Head. Anat. Anz. Bd. XV. 1899.
10) —— Regeneration in Planarians. Archiv f. Entwickelungsmech. Bd. X. 1900.
11) —— Regeneration in Tubularia. Archiv f. Entwickelungsmech. Bd. XI. 1901.
12) Parke, H. H., Variation and Regulation of Abnormalities in Hydra. Archiv f. Entwickelungsmech. Bd. X. 1900.
13) Peebles, F., Experimental Studies on Hydra. Archiv f. Entwickelungsmech. Bd. V. 1897.
14) —— Experiments in Regeneration and in Grafting of Hydrozoa. Archiv f. Entwickelungsmech. Bd. X. 1900.
15) Rand, H. W., Regeneration and Regulation in Hydra viridis. Archiv f. Entwickelungsmech. Bd. VIII. 1898.
16) —— The Regulation of Graft Abnormalities in Hydra. Archiv f. Entwickelungsmechanik. Bd. IX. 1899.
17) Trembley, A., Mémoires pour servir à l'histoire naturelle d'un genre de polypes d'eau douce, à bras en forme de cornes. Leyde 1744.
18) Van Duyne, J., Über Heteromorphose bei Planarien. Pflüger's Archiv f. gesammte Phys. Bd. LXIV. 1896.
19) Wetzel, G., Transplantationsversuche mit Hydra. Archiv f. mikr. Anat. Bd. XLV. 1895.
20) —— Transplantationsversuche mit Hydra. Archiv f. mikr. Anat. Bd. LII. 1898.

Growth and Regeneration in Planaria lugubris.

By

T. H. Morgan.

With 14 figures in text.

Eingegangen am 31. Mai 1901.

In my paper on Regeneration in Planarians (1900) a number of experiments were made on a form designated as Planaria sp.? Dr. WM. WOODWORTH has kindly indentified the species as P. lugubris. The present account deals almost exclusively with this form. The experiments were made from October to March on material collected in the autumn in this locality, and kept during the winter in aquaria. The work was undertaken primarily to study the effect of feeding and of starving on regenerating worms. A number of other experiments were also made, which, in the main, supplement and carry further the results of my previous work.

Since my last paper appeared, BARDEEN (1901) has published an account of some experiments on the regeneration of P. maculata. The results of his work that are new deal more particularly with some of the internal changes that take place during regeneration. BARDEEN states that »the earlier investigators discovered and pictured most of the remarkable phenomena of regeneration in the planarian that have been described by more recent researches«. It may appear from this that the recent work of VAN DUYNE, RANDOLPH, VOIGT, LEMON and myself has only confirmed the results of the older experiments, but I venture to suggest that something has been added that was not known before. It may not be, therefore, out of place to state here concisely the relation of the newer to the older work.

VAN DUYNE's experiment of producing two heads and two tails by splitting the worms partially into two pieces was carried out by

Faraday in 1832, who obtained in this way a two-headed form. A worm with four or five heads was obtained by Van Duyne that »probably were from cutting the original animal in the longitudinal line«. The three cases of heteromorphosis described by Van Duyne, and represented in his Figs. 3, 4, 5, have probably a different interpretation, for although the heads arose at the side, even when an old head was already present, yet this is a different process from the classical cases of heteromorphosis discovered by Loeb in Tubularia, etc., or that of Bonnet's in Lumbriculus cited by Loeb. It is probable in the light of more recent discoveries that several distinct kinds of heteromorphosis exist in some of which the polarity is reversed, as in the first cases described by Loeb, in others not, as in the case of an antenna in place of an eye, and still other so-called cases cases of heteromorphosis may be quite different phenomena. This is illustrated in one of Van Duyne's experiments referred to above (Fig. 3), in which, when the worm is almost completely split in two, new heads appear in the anterior angle between the halves. These heads belong, as I have tried to show, to the anterior end of the new tissue along the side; and have the same orientation as the rest of that side, that is, they appear at the anterior end of a piece incompletely cut in two. They are, therefore, not cases of heteromorphosis like those of Tubularia, Antennularia, Lumbriculus, etc. On the other hand we have a case of true heteromorphosis when another head develops on the posterior end of a head-piece of P. lugubris. If, therefore, on the same worm we find a case of true heteromorphosis we only create confusion, if we attempt to extend the term to include a different phenomenon.

Randolph ('97) showed more precisely than had been done before the mode of regeneration of pieces from different parts of the body of Planaria maculata, and of pieces of different shapes. The result of special interest that is recorded by Randolph is that longitudinal pieces, that represent half, or more than half of the body, replace during regeneration as much as is missing; while pieces less than half the breadth of the old worm do not replace the missing part, but produce at the side as much new tissue as corresponds to their own breadth. It was also shown that worms with two pharynges could be made by splitting the anterior end in half as far back as, and into the region of the old pharynx.

My first paper in '98 included the following results that were, I believe, new. (1) Cross pieces of P. maculata change their form in producing new worms; a cross-piece becomes longer and narrower in the new worm, i. e., a part of the change takes place in the old tissue (morphallaxis)[1]). 2) That if the head is cut off it decreases in size as the new, smaller worm is formed, until it has reached a proportionate size. 3) That there is a minimal size below which pieces do not regenerate. I estimated this size as about $^1/_{100}$ of the worm. 4) That in triangular pieces, with the long end of the right-angle triangle forward the new head (the region of most rapid growth) arises at the side of the anterior end, although the old tissue is less in amount there than at any other point of the cut-surface. This shows that the rate of growth does not depend on the amount of old tissue from which a new part arises. 5) That narrow pieces from the side often produce a head at right angles to the cut-surface, using up all the new material there present to form the new head. 6) That a new head is not formed in the old tissue if the anterior end is closed by the meeting and fusing of the old tissue. 7) That in P. maculata the new pharynx develops, in posterior pieces, in the old tissue. 8) That if cross-pieces are taken from different levels the new pharynx appears at the posterior end of pieces taken anterior to the old pharynx; in the middle of pieces taken through the old pharyngeal region; and in the more anterior end of the old tissue of pieces posterior to the old pharynx.

Voigt ('99) showed that if the anterior end of a planarian is split in two as far back as the region behind the pharynx, and then one half be partially cut off transversely from the inside, that a new tail develops at the posterior part of the latter half, where the cross-cut joins the longitudinal one. Voigt repeated an experiment of Dalyell's, and obtained, by making an oblique cut in the side of the worm, a new head at the side, if the cut was directed posteriorly, and a new tail if the cut was made anteriorly. In Polycelis he found that if, after the oblique cut had been made, and regeneration had begun, the piece then re-united with the body, a row of eye-spots appeared along the line of union.

Lemon ('99) showed that narrow strips partially cut from the side of the planarian, or from the inside of a longitudinal cut, produce a new head and even a new pharynx. The new part often turns backwards, and resembles a heteromorphic formation. These he calls buds, or cases of pseudoheteromorphosis. The experiment is practically the same as that of VOIGT. Certain results are given to show that an injury to one part of the body produces an abnormality, an additional eye, in another part. RANDOLPH had obtained a similar result.

---

[1]) The word should be spelled as above, and not morpholaxis as I have used it before. The word is from the two words μοϱφή and ἀλλάττω.

In my papers on Bipalium (1900) and on Regeneration in Planarians (1900) the following points were established: 1) That in longitudinal pieces less than half the width of the old worm the new tissue increases at the side until it is equal to the old, as RANDOLPH had shown, but further that if the worm is not fed the old part decreases as the new part increases, so that the width of both new and old remains about the same as that of the piece when first removed. If the piece is fed, the old part does not decrease in width, but remains about the same as at first, and may subsequently enlarge as the new worm grows bigger. 2) That in a piece with an anterior oblique surface the new head arises at the most anterior part of the oblique edge, that is at the side of the piece. Similarly the tail arises from the most posterior part of a posterior oblique surface. 3) That if, after removing the head, an oblong piece is cut from the middle of the body, one, or two new heads develop at the remaining anterior cut-surface, the number depending upon the extent to which the sides have fused. In other words the new material at the cut-edge is at first totipotent, and whether it forms one, or two heads depends on whether it is continuous or not from side to side. 4) That if at two levels two half cut-surfaces are exposed a head develops only on the more anterior; the two half-surfaces being connected by a line of new tissue. 5) That if a double-tailed form has one tail cut off near to the body a new tail does not develop, but if cut off further from the body a new tail develops. 6) That if the head of P. lugubris is cut off just behind the eyes a new heteromorphic head is produced at the cut-edge. 7) Experimental evidence was given to show that in Bipalium elongation in the old part of a cross-piece is the main-factor in the production of the new worm. 8) That if a worm is cut in two lengthwise from the posterior end, as far forward as the region between the eyes, each half may produce a half-head on the inner side. 9) That if after the formation of new material at the anterior and posterior ends of a cross-piece the piece is split longitudinally into halves, the new half-material may produce a half-head, that later completes itself at the side. If the same operation is carried out on a less advanced piece, each piece produces at first a whole head out of the new material. The most natural interpretation of this result is that the new material is at first totipotent and more or less indifferent, but soon becomes predisposed in a definite direction.

Bardeen has confirmed the following results. 1) The lateral position of a head and tail on oblique pieces. 2) The elongation of the old part to form a new worm (morphallaxis). 3) The development of a heteromorphic head. 4) The formation of one, or two heads in the anterior angle of a partially divided worm 5) The lack of power of pieces cut off in front of the eyes to regenerate. 6) The development of two heads by splitting the anterior end. 7) The development of a lateral head from an oblique cut in the side. The new points that Bardeen has made out are: 1) The fusion of the branches of the digestive tract to locate the region of the formation of the pharynx. 2) The formation of the new nervous system as an outgrowth from the old one. Bardeen has described also at some length some of the processes of motion, feeding[1]), sensation, etc. in planarians.

In regard to the formation of the new nervous system I have seen some evidence in Bipalium and also in Planaria lugubris to show that the new nerves and brain may arise from the general mesenchyme, as described by Flexner ('98). It is a difficult point to decide, but there are certainly indications of there being new cells added to the new nervous system that have not come directly from the old ones. I am inclined to think also that in certain cases the tissue that is to form the new pharynx appears before any such central sac, as that described by Bardeen, has been formed in the digestive tract. Bardeen's view that this central sac is a center from which the influences, that go to produce the anterior structures, radiate, is in my opinion far too naïve a view to account for the results, and, moreover, the development of the lateral head in an oblique piece like that shown here in Fig. 10 makes this view improbable. In this case the new head had developed at the side in a region where there were present only isolated pieces of the digestive tract. Later when the branches from the two sides had united, and a communication with the pharynx had appeared this region lay far removed from the new head. Bardeen's view that the lack of development of pieces cut off in front of the eyes is due to the absence of a part of the old digestive tract is very probably correct, although it may as well be true that it may be due to the part of the digestive tract that is left being too small to produce a new one.

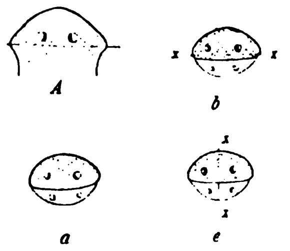
---

[1]) Bardeen's statement that P. maculata does not respond to the presence of food in its vicinity unless the food is brought in contact with the worm is undoubtedly wrong, as the worm responds freely to certain kinds of food — a piece of a fly, for instance, — that may be far away from the worm.

Heteromorphosis in Planaria lugubris.

When the head is cut off just behind the eyes another head often develops on the posterior end of the old head, Fig. 1 *A*, *a*, as I have shown in my last paper. I repeated this operation a very large number of times in the hope of discovering what special conditions lead to the result. Many of the pieces die, especially if the water is not perfectly clean. In a few, very successful cases however nearly 50 per. cent. of the pieces produced another head. The other pieces died, or failed to regenerate at all, or made a new tail. The latter result takes place when the piece is cut off too far posteriorly, Fig. 2 *a*.

Fig. 1.



It seemed to me that the formation of a posterior head might be due to the cut passing through the old brain. I made sections of several pieces just after removal, and also of some of the fully formed amphicephalic pieces to test this view. It was found that the pieces had been cut off behind the region of the brain, and that when the new head had developed the two nerve-cords from the old brain were continuous with two cords that came from the brain in the new part. In other words, the result is not due to the cut passing through the old brain. The sections were also examined to see if the pieces contained any of the old digestive tract. In this planarian the branches of the digestive tract extend almost to the eyes, and a single median branch extends forward between the eyes. It seemed possible that this median tube, if in the head-piece at the time of its removal, might be lost by being forced out of the cut-surface. The absence of a part of the digestive tract might at least account for the failure of the new tissue to produce a new tail, and some other factor might then determine that the new part produces a head. The sections showed, however, that in all cases a part of the old digestive tract was present and in some cases occupied a large part of the middle of the interior of the piece. The result, therefore, is not connected with the loss of the part of the old digestive tract, and the cause of the phenemenon remains as great an enigma as before.

We can only ascribe it vaguely to some kind of influence of the old head-tissues on the new part, or else to the kind of cells of which the new part is composed.

Several of these double headed pieces were kept as long as twenty-three days in order to see if a pharynx might develop between the two heads, but this did not take place.

In a number of cases the double-headed pieces were cut in two in the middle by a cross-cut as shown in Fig. 1 *b*, *x*—*x*, in order to see if either piece would produce a new tail, or another head, but the pieces died without regenerating again. A few pieces were cut in two longitudinally as shown in Fig. 1 *c*, *x*—*x*. I wished to find out if the double half-pieces would regenerate laterally, and if so, whether the halves would complete themselves, or possibly produce a new third head at right-angles to the two half-heads. The pieces closed in, and in doing so brought the half-heads more, or less in contact at the cut-sides. Most of the pieces failed to make any new tissue, or made only a little wedge-shaped piece, in which, in one or two cases, a rudimentary eye developed.

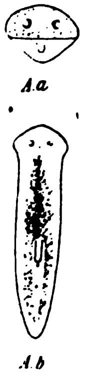
In my previous work on this form I had found several times, that when a small piece was cut from the tail-end a pointed, new part appeared, that did not become a head. It seemed possible that this might be a heteromorphic tail. I did not publish this result because, in the absence of any specifically characteristic structure in the part, I could not determine whether the new growth was really a tail, or only an undeveloped head. A repetition of this experiment shows that the structure is an undeveloped head. In this worm it is usual for the tail-pieces to regenerate a long, anterior new part before the eyes appear — as shown in Fig 7 *e*. If the piece is small so that an insufficient amount of new material is formed, the new part does not reach a size sufficient to produce a head, hence the appearance of a two-tailed piece.

A large number of very narrow cross-pieces were cut off just behind the head in order to see if two heads would develop from such pieces, but I never found this to occur.

The Formation of a Sexually Mature Worm from a Piece without Reproductive Organs.

It has been stated that many of the pieces cut off just behind the eyes produce a new tail. These head-pieces have been cut off some distance in front of the ovaries, that lie in the anterior third of the body. By feeding the pieces, I succeeded, after a number of failures, due to death and disease in the pieces, in bringing a few to the average size as shown in Fig. 2 *A a*, *b*, and later to full grown worms. These worms developed in the course of several months. They had each a reproductive pore which could be seen on the ventral surface. One was killed, and cut into sections. A study of the latter showed that the worm was normal in every respect, and that the reproductive organs including the ovaries were fully developed. The other worms laid eggs. Since the pieces, came from a part of the body some distance in front of the region of the old reproductive organs the result shows that new germ-cells can develop from the somatic tissues, or at least from cells not included in the old reproductive organs.

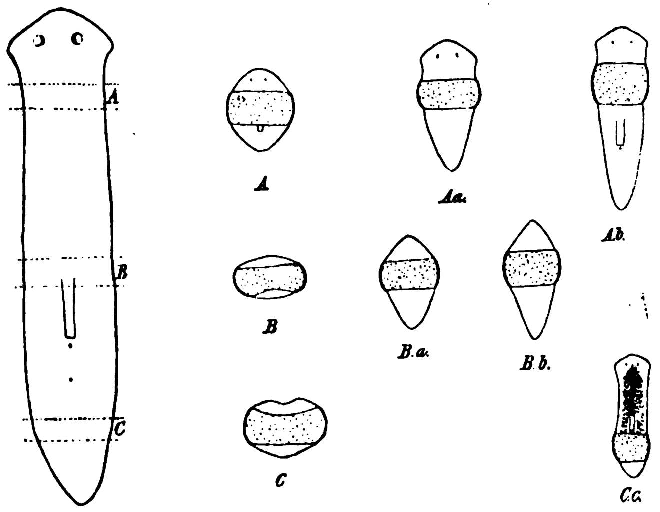
Fig. 2.



Method of Regeneration of Cross-Pieces from the Anterior, Middle and Posterior Parts of the Body of Planaria lugubris.

It has been shown in preceding papers that the position of the new pharynx in cross-pieces is connected with the part of the body from which the piece has been taken. I had often observed that the amount of new tissue produced by the anterior and posterior cut-surfaces, as well as the extent of elongation of the old part, is also connected with the region of the body from which the piece has come. In Planaria maculata, and in Bipalium, the elongation, that takes place in the old part, appears to correspond to this general law. In Planaria lugubris the elongation of the old part does not take place to anything like the same extent, and the change is more largely due to the development of new tissue at the cut-ends. If we compare the development of a narrow cross-piece of P. lugubris from the region just behind the head. Fig. 3 *A*, with the regeneration of a similar piece from the middle of the body Fig. 3, *B*, and with one from the posterior part, Fig. 3 *C*, we find important differences take place in the regeneration of the pieces. The anterior piece, *A*, produces little more than a new head at its anterior end, Fig. 3 *A*, *a*, and the new head is not carried further forward by the subsequent growth of new material behind it, Fig. *A*, *b*. The same piece produces at its posterior end a new tail, containing a pharynx, and if the piece is fed this new part continues to grow longer, carrying the pharynx more posteriorly until an equilibrium is reached between the new and the old parts, plus the new head. After this the entire piece will grow larger if the feeding is continued. If the new worm is not fed the tail grows longer, but more slowly, and the old part continues to get narrower from side to side. The process goes on until the typical proportions of the parts are attained, that is until the new pharynx has been carried to its proportionate distance behind the head. If the piece is now fed it will enlarge at a proportionate rate in all its parts.

Fig. 3.



The middle piece, *B*, produces a new head at the anterior end. A new tail appears at the posterior end, and a new pharynx appears in the middle of the old part in the old pharyngeal chamber[1]. The new parts at the two ends grow longer at about an equal rate; the head being carried forward as the result of new tissue appearing behind it. I have not made out where the region of growth lies in the posterior part, but to judge from what happens in the more anterior pieces, where the pharynx is carried backwards by growth in front of it, it seems probable that the new growth in this case also is near the base of the tail, or throughout the entire new part. If the piece is fed the two new ends grow rapidly, and the middle part remains about the size it had been reduced to as a result of the first formation of new material at the two ends, or it may grow larger. If the piece is starved the old part is further reduced in size until an equilibrium is established. A smaller worm results.

---

[1] The middle piece shown in the figure did not produce a new head at the anterior end, although as shown in Figs. *Ba* and *Bb*, much new tissue developed. The pointed anterior end appears in this case to represent an undeveloped head and not a heteromorphic tail.

The posterior cross-piece, *C*, produces a head at the anterior end, although it is very noticeable in this form (but not in P. maculata), that more new material appears before the head differentiates (and there is a delay of several days), as compared with the development of more anterior regions. A short tail appears at the posterior end of the piece. A new pharynx appears just behind the head in the new part where the new and the old parts are continuous. It lies, at first, almost in the new head. New tissue continues to develop in the anterior part, and as this growth takes place mainly between the head and the pharynx the former is carried further forwards. If the worm is not fed, the old part decreases, especially in breadth, as the new part develops. If the piece is fed, the new parts grow faster, and the old part loses less, or may remain the same size.

These results show that cross-pieces from different parts of the body regenerate in different ways. The old piece acts as a barrier, and holds the same relative position in the new worm that it held in the old. The nature of the old piece is the determining factor in the subsequent growth in the new part. There is nothing, as far as could be foreseen, that would prevent an anterior piece growing with equal rapidity at the two ends in order the reach the symmetrical condition, but this does not occur. On the contrary the new pharynx is carried backwards away from its first position. In a posterior cross-piece the conditions are somewhat different, since the position of the new pharynx just behind the new head makes it necessary that the new material be laid down between the head and pharynx in order that they shall stand in their proper relation to each other. It is important to note that in both anterior and posterior pieces the region of growth lies in front of the pharynx.

The method of regeneration in Planaria lugubris throws some light on the changes that take place in P. maculata. In the latter, and even more strikingly in Bipalium, the changes that take place in a cross-piece involve especially the old part. After the first formation of new tissue to make the new head and tail the old part elongates throughout, becoming narrower at the same time. This change appears, nevertheless, to be regulated by the nature of the piece in much the same way as in P. lugubris. Thus in a cross-piece from the anterior end of the worm the elongation in the old tissue is, for the most part, in the posterior region; and in a cross-piece from the posterior end the elongation is greatest in its more anterior part, etc.

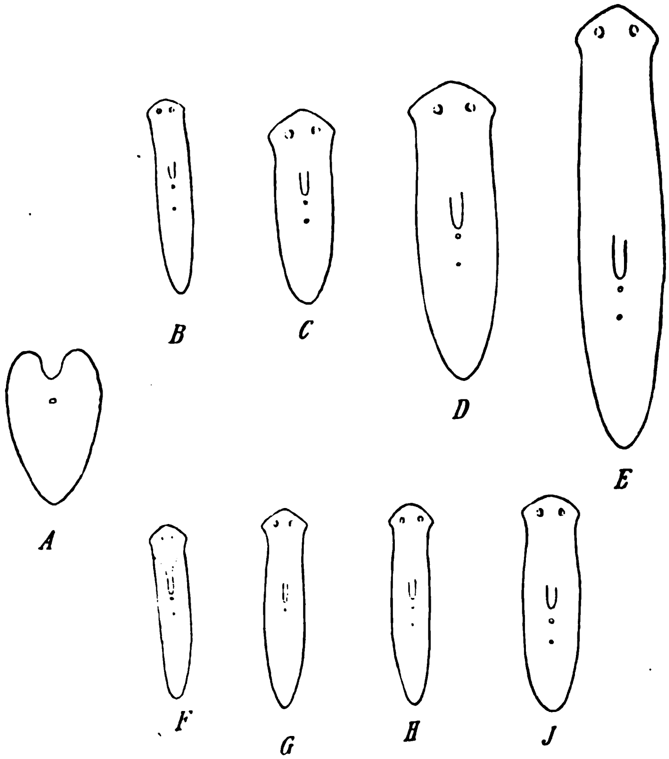
When the tail-end behind the old pharynx is cut from P. lugubris the new pharynx comes in, as has been shown, at the edge of the new tissue at the anterior end, and, therefore, almost in the new head. It seemed that this might be due to the inability of the pharynx to develop in the old tissue, as it does in P. maculata, and that if a posterior cut-surface also existed the new pharynx might appear in this new tissue at the posterior edge rather than at the base of the new head. A number of pieces were cut off, posterior to the genital pore, similar to those shown in Fig. 3 *C*. In every case the pharynx appeared at the anterior edge, and quite near the new head. The old piece has acted as a barrier preventing the pharynx from appearing at its proportionate distance from the new head, as would be more nearly the case if it developed at the posterior cut-edge. The result shows that the ordinary idea of polarity does not apply here, since a posterior structure — the pharynx — appears at the anterior end of a piece if this piece comes from behind the pharynx in the old worm. It is not simply a question of anterior and posterior ends of the cross-piece, but of the relation that this piece held to the parts of the original worm, that determines its action; and this is all the more important, since it has been shown, that from any part of the piece any part of the new worm may arise.

The Effects of Feeding and of Starving on the Regeneration of Pieces.

In the preceding pages it has been shown that by feeding the newly formed worm the old tissue can be protected. Whether the protection is brought about by the growth of the old part keeping pace with the loss of cells, or of material, sent into the new parts, or whether the new parts, being well nourished, do not draw on the old part for cellular or for food material is not clear from the results of my earlier experiments. The following results throw some light on this question.

The posterior ends of several worms of about the same size were cut off just in front of the old genital pore. The pieces were seperated into two lots, one of which was kept without food, and the other supplied with food as soon as the new pharynx had developed. The distance from the genital pore to the posterior end was 3 mm, and the pieces measured each about $3^1/_2$ mm long by 2 mm broad.

Fig. 4.

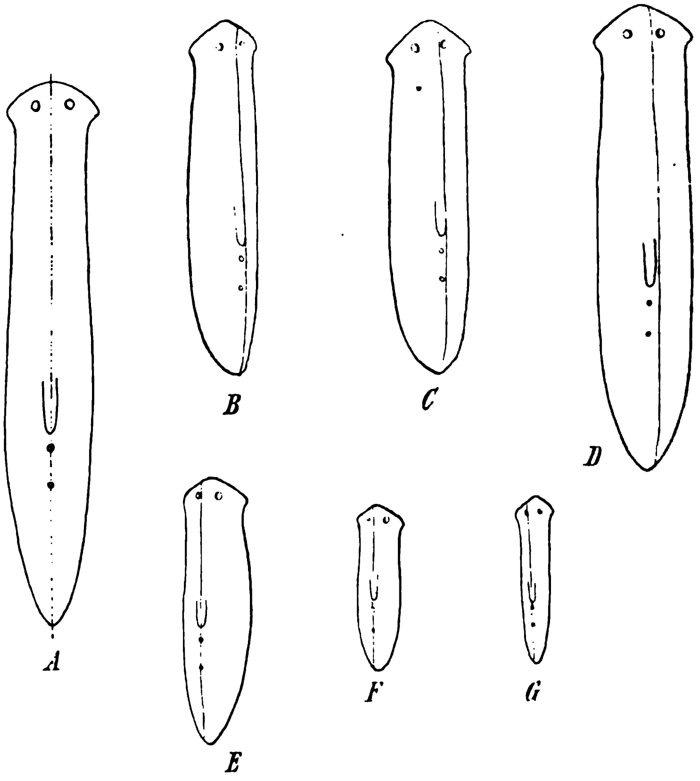


Nineteen days after the operation the worms appeared as shown in Figs. 4, *B* and *F*. The pieces have made a new head and pharynx and have diminished nearly a half in breadth; but the distance from the genital pore to the posterior end is about the same as at first. One of these worms was fed, Fig. *C*; the other kept without food, Fig. *G*. After nineteen days the two worms had reached the condition represented in Figs. *D* and *H*, and eight days later that shown in Figs. *E* and *J*. The larger of these last two figures represents the worm as fully grown. The starved piece also appears somewhat larger than in the last figure, due either to the piece being flatter when measured, or possibly to its having found some food.

The results show that there is a primary loss of material in the old part during the formation of the new head and pharynx; that the loss in almost entirely in breadth and height, the length of the old part remaining as at first. After feeding a piece becomes broader, and also continues to grow in length especially in the region between the pharynx and the head. The starved piece grows only a little longer anteriorly, but even after two months the anterior end had not reached the typical length, i. e. the posterior end was too long for the anterior end. The most surprising fact is that the starved piece loses very little of its original length, but half its breadth. The slight loss in length may be accounted for by the general dwindling of the old part due to lack of food, but the very great diminution in breadth must be due to the loss of material that goes, either directly as cellular material, or indirectly as food material, to the anterior part.

In another experiment a worm (measuring $12 \times 1^{9}/_{10}$) was split in two pieces along the middle line on Oct. 3, Fig. 5. One piece was fed for the first time on Oct. 13, and later at intervals of a few days. The other piece was kept without food. The two pieces were measured on Oct. 17, and are represented in Figs. *B* and *E*. The piece that was fed measured $8 \times 1^{3}/_{4}$. It had lost one third its length, and the new and old parts together were broader than the old half. The starved piece had decreased one-half in length, but was about as broad as the last. This apparent increase in breadth is largely due to the old part remaining in a more contracted state, even when moving forward, than when it formed a part of the original worm. The new tissue that appears along the cut-side arises while the piece is at rest, and in a contracted condition. When the new worm is irritated, so that it moves, the new part does not permit of the fullest possible extension of the old side, which appears, therefore, broader. This seems to be the most probable interpretation. After another fourteen days the two pieces appeared as shown in Figs. *C* and *F*. The piece, that had been fed every few days, has not increased noticeably in length, but is broader than before. This increase in breadth is in part due to the growth of the new region, but also to some extent to growth in the old part. This is seen from the fact that although the width of the new worm is as great as that of the original worm, yet the pharynx, that appeared at the edge of the old part, still lies excentrically. The starved piece is only one-third its former length, and about one-half its width. The pharynx lies in an excentric position, and relatively even more excentrically than in the fed piece. This means that in the starved piece there is not only absolutely less new tissue formed, but even relatively less than in a piece that is fed. The later condition of these two pieces is shown in Figs. *D* and *G*. The larger piece measures $10^1/_2 \times 2^1/_8$. It is nearly as long as the original worm, and somewhat broader. The latter is due to the abundant supply of food, for had the original worm been overfed, it too would have gained more in breadth than in length — to a certain maximum limit beyond which it could not be forced. The pharynx is near the middle of the piece, although slightly excentric even at this time. The smaller piece is represented in Fig. *G*. It measured $4 \times {}^5/_8$ and is, therefore, not much more than half as wide as before. The loss

Fig. 5.



seems to have been mainly in the old part, so that the pharynx appears more nearly symmetrical. The new part is about as wide as it was before.

These results show that by feeding the new worm the old material is protected, and the new material grows faster than in a worm without food. The new pharynx appears along the edge between the old and new parts, and holds that position throughout all subsequent changes; the same is true for the genital pore, and for the eyes, as well as for the central part of the digestive tract. This gives very strong evidence in favor of the view that after the first formation of cells along the cut-edge the subsequent changes are due to growth in the new part, rather than to the migration of cells into the new regions. From this point of view the decrease in the old material in starved worms is not due to cell-migration, but to loss of substance. The growth of the new part must be due to nourishment received from the old part.

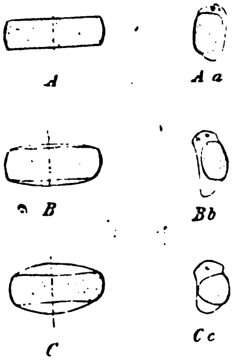
It is a well known phenomenon that whole worms kept without food decrease rapidly in size until they are as small, or smaller than the starved half-pieces shown in Fig. 5 *G*. The most important fact in regard to the regeneration of the starved half-pieces is that the new part grows at the expense of the old part; or put differently; one half of the body that itself is lacking in food is used as food material for the other incomplete half. The decrease in the length of the new worm may account for a part of the increase in breadth of the new part, but the results show, I think, that the greater part of the increase in breadth is due to material taken from the other half. This transportation of food substances to regions of more active growth, where the changes are going on that produce a symmetrical form, is most remarkable. It indicates that growth processes may control the distribution of food material in the animal. Growth, from this point of view, is not altogether the result of the presence of food material, although it may take place at a more rapid rate if an abundance of material is present, but the growth determines the relative distribution, or deposition of the material that is present.

This same relation between growth in the old and in the new parts seems to hold for all kinds of pieces, so that it will not be necessary to give any further description of the process in other cases, except in so far as the results bear on each particular experiment.

Regeneration in Half Cross-Pieces.

These experiments are in part a repetition of a similar one on P. maculata described in my last paper. They have, however, given more definite results. Short cross-pieces were taken from the anterior end about midway between the head and pharynx. Some of the pieces were split in two at once, Fig. 6 *A*; others after three days, when a small amount of new material had developed at the ends, Fig. 6 *B*; and others an the following day before the eyes had appeared, Fig. 6 *C*. After seven days the first pieces had produced each a new head with two eyes, as shown in Fig. 6 *Aa*. It will be seen that the head lies somewhat towards the cut-side of the piece, i. e., its median plane passes just within the edge of the old piece. The inner eye appeared nearly always a little later than the outer one. The new pharynx also lies towards the inner side of the posterior part.

Fig. 6.

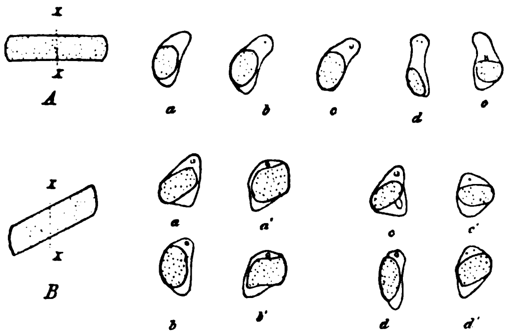


The second lot also produced, after eight days, two eyes, but the inner one was less well developed, Fig. *Bb*. The third series formed at this time one eye, the outer one, in each piece. A day later the other eye appeared in two pieces, but in four pieces only one eye was present. Two days later all, but one, had two eyes, and on the following day the second eye appeared in the last piece.

The results show that if the cross-piece is cut in half when the new material is not well developed a single head with two eyes is produced, but if the same operation is made a little later, only one eye develops at first, and the other develops a day or two later. This appears to mean that in the latter case the new head has begun to be formed, and the half-piece continues to differentiate into a half-head. The other half is subsequently regenerated laterally. When the new material is first produced it is indifferent, and from it may be produced a single head, or two new heads if it is cut in two; but a little later the differentiation has gone so far, that if the material is divided it continues to differentiate into a part of the whole. It may be, of course, that even at the beginning the material is differentiated to a slight extent; but the material is still capable of rearrangement.

The new head in these cross-pieces lies towards the inner side of the piece, i. e., nearer to the old middle line than to the middle of the piece itself. One of the factors that determines the position of the new head may seem to be the old middle of the piece, since the new middle line of the head tends to correspond with that of the old part. Two possible explanations of this suggest themselves. The new material is thicker near the inner side of the piece than at the outer side, and if the amount of material is a factor in the result, that is, if the new head tends to assume the middle of the mass it will lie nearer to one side. On the other hand the development of the head at the outer side of an oblique piece appears opposed to this view, and also the less development, or later development of the inner side of the head. We might assume that the position of the central part of the digestive tract at the inner side of the piece has a determining influence, but this seems improbable also in the light of the result from oblique pieces. Neither of these views, therefore, seems to give a satisfactory explanation of the result. Another possibility will be discussed later. Since these half cross-pieces came from the anterior end of the worm it seemed to me that possibly half cross-pieces from the posterior end might behave differently: that is, the new head might assume a more central position at the anterior cut surface, because the influence of the old part might be different in a region further removed from the old head. Moreover, since there are two lateral branches to the digestive tract in this posterior region the larger part of the old digestive tract will lie nearer the middle of the half-piece than in the anterior pieces. Posterior pieces produce at first a large amount of new material before the new head develops, Fig. 7 *A a.* Six days after the operation a half cross-piece had not developed eyes, although a pharynx was present, Fig. 7 *a.* Two days later one eye had appeared in one piece, but the second eye did not appear in this piece until the thirteenth day, Fig. 7 *d.* In one piece, Fig. 7 *e*, no eyes were present on the ninth day, but later first one, then the other appeared. The results are practically the same as on the more anterior pieces, except for the greater length of time that elapses before the first eye appears, and the larger amount of new material that is produced. The results seem to show that the asymmetry of the new head is not due to the digestive tract, or connected with the nearness of the piece to the head of the old worm. The most abvious fact is that the inner side of the head develops less rapidly in all these pieces than does the outer side. Now the new, inner side of the body, represented by the new material at the side, is also less developed than the side represented by the old part. The piece takes this condition, if I may so speak, into consideration, and the development of the inner side of the head is held in check by its relation to the rest of its own side. This is the only interpretation that I can offer of the result, and as in many processes of regeneration it refers the result to that inscrutable phenomenon that we call the organisation. Even if at present we can not grasp the meaning of this organisation, it seems to me more satisfactory to find that the phenomenon belongs to this category than to give it a more simple, but possibly an erroneous explanation.

Fig. 7.

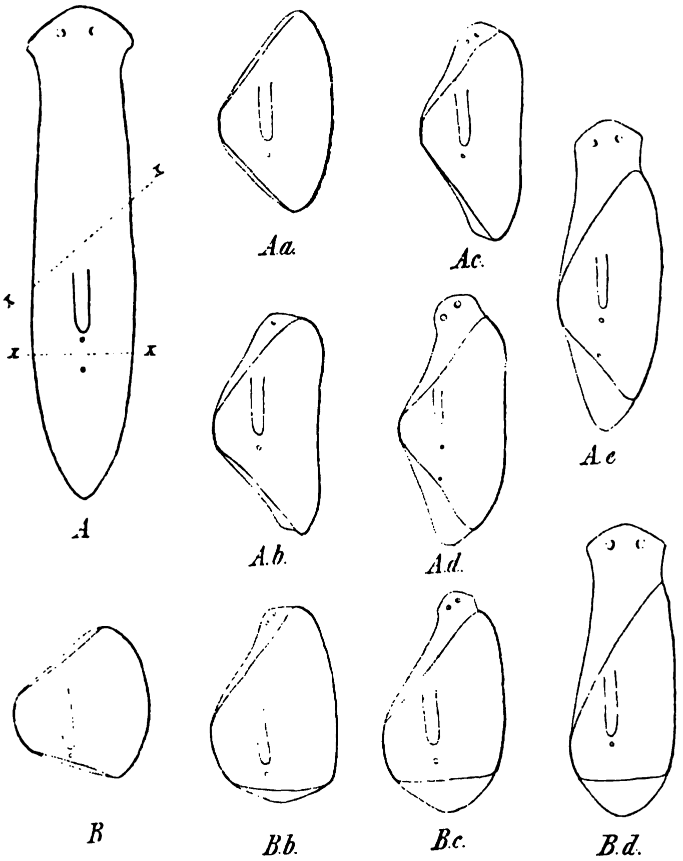


A number of similar experiments were made with oblique half cross-pieces, Fig. 7 *B*. I have previously shown, in four species of planarians, that the new head appears on an oblique surface at the side of the anterior part, rather than in the middle of the piece. If an oblique cross-piece is cut into two half-pieces as indicated in Fig. 7 *B*, *x*—*x*, we should expect to find that in the right-hand piece the head would appear on the outer right-hand side, but since there has been shown to be in half cross pieces some influence that brings the head towards the inner side we should expect to find the new head, adjusting itself to both tendencies, appearing about the middle of the anterior edge. In the left-hand half-piece both of the former influences would tend to bring the new head towards the inner side. The figures show, Fig. 7 *B a*, *a'*, *b*, *b'*, etc. that the anticipated results follow, although owing to the change in shape of the old part it is not always clear just where the new head arises. In both cases a single eye first appears — always on the outer side of the piece. In the right-hand pieces the head lies nearly in the middle of the anterior edge; while in the left-hand pieces it arises at the inner angle.

Regeneration from an Oblique Surface.

It seemed to me that the development of a new head on the side of an oblique piece, rather than in the middle, is such an exceptional phenomenon that it would be well worth while to give it a more thorough examination. I wished to make out more particularly how such a piece regains its symmetry. This change, it was found, could be best studied if the piece contained a part of the old pharynx and genital pore that serve as landmarks and prevent mistakes that might possibly arise from a change in shape of the piece. It was also found better to keep the pieces well fed, so that the old part keeps more nearly its former size. This may of course alter somewhat the

Fig. 8.

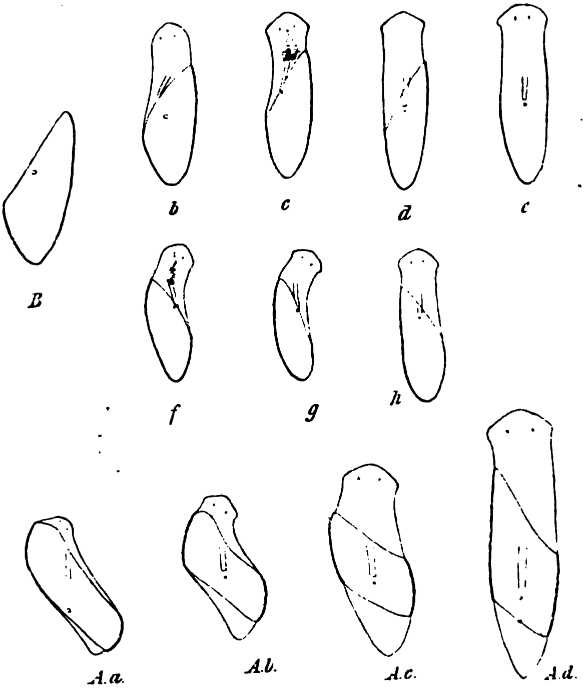


result. If the middle piece of the worm is cut out as shown in Fig. 8 *x—x*, *x—x*, having an anterior oblique edge, and a posterior square edge, the old pharynx lying in the middle of the piece, the following changes take place. The edges of the cut-surfaces bend in, and the piece contracts, and becomes more rounded, so that it is broader than the original worm, Fig. 8 *B*. Owing to the greater contraction on the longer, right side the old pharynx appears to lie nearer to the opposite side. The new material that appears along the cut-edges grows fastest at the right side of the anterior cut-edge, and in the middle of the posterior edge, to give rise to the new head and new tail, Fig. *Bb*. The head is small, and its median axis stands at right angles to the cut-surface. The old pharynx extends forward to the middle of the anterior edge, and bends over somewhat towards the head. Its position shows, however, that the new head really arises far to one side of the piece. The inner eye is sometimes less developed than the outer, and this appears to be connected with less development of the inner side of the new head, and of the region to the left of it. The new head grows forward, the greatest amount of growth occurring on the inner, less developed side. It assumes in consequence a more anterior position. The head becomes larger, and broader, and, in consequence, its middle plane comes to correspond more nearly with the middle line of the old part, as shown in Fig. *Bd*. When the head has reached its full size it lies in a symmetrical position in regard to the old part. The worm was fed occasionally so that the old part lost very little in breadth.

If a piece is removed by two oblique cuts that are inclined towards each other as shown in Fig. 8 *Aa*, the changes are very similar to those just described. The head as well as the tail arises at the side of the piece, that is in this case at the right side of the anterior and posterior cut-edges. The series of Figs. *Aa—Ae* shows the successive stages in the regeneration of this piece. The figures represent the worm as seen from the ventral side, while crawling on the under surface of the top of the water.

If a piece is removed by two parallel oblique cuts as shown in Fig. 9 *A*, the subsequent changes are like those represented in Figs. 9 *Aa—Ad*. Although the piece was fed the old part became narrower, and somewhat longer, but the main change is in the new material at the anterior and posterior ends. The shifting and straightening of the new head are brought about by its enlargement and by its more rapid growth on the inner side. The old pharynx bends somewhat towards the new head in the earlier stages. The posterior end also reaches its symmetrical position in the same way as does the head. This piece was fed. If the piece is starved the new head and tail grow less rapidly and the old part decreases in breadth, so that a smaller narrower worm is formed.

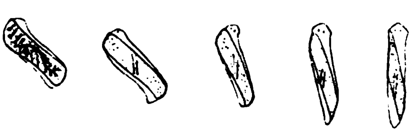
Fig. 9.



A very interesting result is obtained when the tail-end is cut off obliquely, the line of division passing obliquely between the pharyngeal and genital pores, Fig. 9 *B*. The new pharynx appears in the new tissue as shown in Figs. 9 *b* and *f*. It extends obliquely across the anterior edge of the old part, and lies almost parallel to the cut-edge. Its posterior end lies in the middle of the old part, while its anterior end extends forward towards the middle of the new head. From the anterior end the middle part of the digestive tract extends obliquely to the middle of the head. This result shows the nature of the new material along the anterior edge, and makes much clearer how the establishment of the later symmetry is brought about. We must conclude from this experiment that the old median line is connected with the median line of the new head by obliquely lying median structures in the new part; as indicated by the position of the pharynx. The new material on the outer side is less developed than on the inner, and the new head bends over towards the less developed side for some time. The later growth is more rapid on the undeveloped side, and this combined with the enlargement of the entire, anterior end establishes the later symmetry, as shown in Figs. 9 *c* to *e* and *f* to *h*. The eye on the less developed side often appears later than on the other side and this supports the interpretation that I have given for the conditions affecting the position of the eyes in the half-cross-pieces.

The lateral position of the new head can not be determined, I think, by the position of the new pharynx, or of the old one, if it is present in the piece, for the central part of the new digestive tract lies often at, or near the old middle of the piece. This is well shown in the oblique piece drawn in Fig. 10. The worm, P. maculata, had been fed (several hours before this oblique piece was cut off) on the head of a fly, and the walls of the digestive tract were colored a bright red, and for several days this color remained so that the branches of the digestive tract could be seen. Three days after being cut off the digestive tract had assumed the form shown in the figure. The cut had been made across the old pharynx. Two branches have united in the middle line in front of the old pharyngeal chamber, but the branches on the left side had not yet united. The greater growth of new tissue at this side marks the beginning of the new head. This result shows, I think, that Bardeen's idea that the middle part of the digestive tract determines the position of the new organs fails in this case to account for the position of the head, and is probably not the determining factor in the result.

Fig. 10.

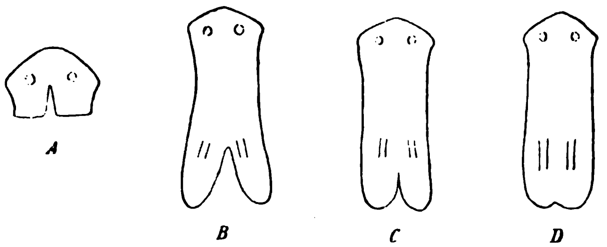


The Effect of Partial Splitting along the Middle Line.

The experiment of partially splitting the worm along the middle line was first carried out by Faraday, and double structures obtained. Van Duyne repeating the same experiment discovered that when the cut extends far forward one, or two additional heads appear at the anterior angle where the sides meet. The same experiment with the same result has been repeated by myself (1900) and by Bardeen. I found further that if the cut extends forward between the eyes each half-head may complete itself on the inner side, and Bardeen has also figured a double worm showing the same condition. More recently I have again obtained one case in which the two halves were united by only a very narrow band of tissue at the anterior end, and in this instance each half formed a new, entire half-head on the inner side. Subsequently the united worms broke apart while crawling.

The question naturally arises as to whether there is any connection between the cases in which each half of the head completes itself, and those in which one, or two new heads arise in the anterior angle. I think that it can be shown that both are the outcome of the same factors. The explanation is an extension of the same principle that I made use of in a former paper (1900) to account for the heads that appear in the angle. According to this view each head is produced at the anterior end of the new side in the same way that a head is produced at the side of a longitudinal piece. It is prevented from turning forward by the old tissue that meets across in front of it. When the cut is extended still further forward, so that the old head is cut nearly in half, the new tissue at the side completes the old head, that is, it carries out a similar process as before, but in some cases completes a half structure already present; and in the other cases when the new part does not extend into the new head, an entire new head is formed at the side. The two results differ in that in one case the new tissue at the side is influenced by the presence of the half head along which it lies, while in the other case the old head has no influence on the result.

Fig. 11.



Another experiment shows, I think, that this interpretation is correct. If the anterior end of the worm is split in two, the two halves being held together at the posterior region, a tail may form on one, or both sides at the posterior end of the new tissue. Voigt and Bardeen have each given a figure showing this condition. It is scarcely possible that the old tail can have any influence on the result and, I think, that there can be little doubt that it is due to the posterior end of the new tissue producing in itself an orthomorphic tail. The case is parallel to the head produced in the angle of the other pieces.

In a few cases the anterior end was cut off and then split partially in two as far forward as the region between the eyes, Fig. 11. The experiment was made to see if new heads would appear in the angle between the sides, when the region behind the angle is quite short. Although the halves were separated again and again they persistently fused together. Most of the pieces produced two pharynges, in some there was one in each new tail, Fig. 11 *B*; in others the two pharynges lay side by side in a single body that was forked behind, Fig. 11 *C* and *D*. These pieces were fed and they grew larger an longer. I tried to determine, if, in these pieces with two pharynges in a single body, the body would be relatively broader than in a normal worm of the same length. This was found to be the case, at least so far as the region showed a double structure. I tried also to determine if the new head on such a body would be twice as broad as that of a normal worm of the same length, but this did not appear to be the case. Since the old head arises from a region in which the structure is not doubled it could scarcely be expected that it would develop to twice the proportionate breadth. A better way of testing this problem is to cut off the anterior end in the region in front of the two pharynges where the worm has to a certain extent a double structure. As most of the pieces of this sort that I had kept for some time became diseased, and died, I could not carry out the experiment, but it does not seem probable that the new head would be broader than a normal head, unless it had a more or less double structure, and I do not think that this would occur in a head forming in the new tissue over the cut-surface.

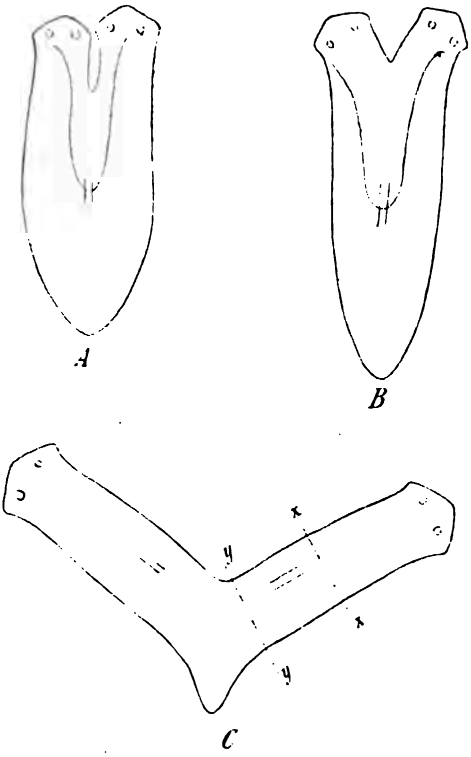
The Size of New Heads of Worms Split in two at the Anterior End.

In the first series of these experiments the head of the worm was cut off, and an oblong piece removed from the middle of the body, as described in my last paper[1]). If the anterior halves are kept from uniting a new head appears on each side, Fig. 12 *A*, *B*. The size of each new head is in proportion to the size of the side-piece from which it arises, as can be easily seen when one side is larger than the other. New tissue may appear where the sides partially fuse behind the double head, Fig. 12 *A*, *B*. The subsequent growth of the new heads depends upon the length of the region by which they are united to the general body. If this region is short the heads remain small Fig. 12 *A*, *B*, in fact they are often not more than half the normal size. If the connecting region is long the new heads become correspondingly larger. Some worms of these kinds have been kept for several months and supplied with food, but those with heads closely united to the body do not produce larger heads, or at least to only a slight extent in proportion to the general growth, and the heads remain practically half-heads. When the heads are united to the body by a larger piece the heads may grow to the normal size. This difference is the result of two factors. First the breadth of the region that unites the head to the body, and secondly the length of the connecting region. The size of the new part is determined, or regulated by the size of its region of union, and if the piece is short it may be held in check by the area of its connecting region; but if the piece is long (i. e., when the connecting region is further away) it tends to assert its own independence, and may overcome the regulative influences of the base. These statements seem to apply to all cases in which double structures are produced by splitting as the following results show.

---

[1]) Pages 90 and 91.

Fig. 12.



If, instead of cutting off the old head and removing a piece from the middle of the trunk, the anterior end is simply split in two, each half will, if prevented from fusing with the other half, produce a new half head at the side, Fig. 12 *C*. Each new head is there made up of half the old head and half of a new one. The question arises whether the new half of each head will enlarge to the full size of the old half, or whether the latter will reduce itself to a smaller size. The results show that a reduction of the old part takes place, which may be accounted for, in part at least, perhaps entirely, by the loss of cellular material to produce the new half.

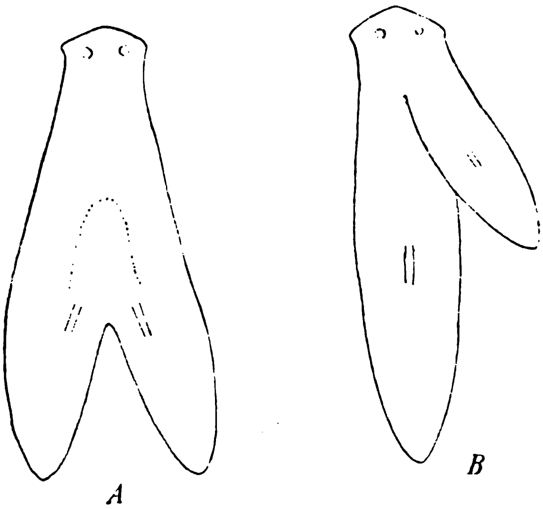
If the worm has been split further posteriorly, and the halves kept from reuniting, the new heads will become larger, Fig. 12 *C*. Each half forms a new side that is smaller at first than half of the original worm, but if the pieces are well fed, each half may grow to nearly the normal or average size, and the head also becomes nearly as large as the head of an average worm. We see here the same changes that have been described in the preceding experiment, and the same interpretation will account for the result.

Another question that arises in connection with these pieces is whether a two-headed worm will enlarge so that its body is twice as big as the normal worm; in which case each head might then become full sized. This does not occur, however, as the figures show, since the single part of the body remains about the normal size. It may appear somewhat broader when a median piece has been cut out, Fig. 12 *A*, since the double structure may to some extent involve the newly formed, common, middle part, or it may appear broader, because the anterior end spreads out somewhat more at the sides than in the normal worm; but, in general, it is clear that the body does not become proportionately twice as broad as it is in normal worms. When the pieces are separated further posteriorly each part, as has been described, may become nearly as broad as the original worm, but the part of the body from which the halves arise remains about the same size as before — except in so far as it tends to spread out to its maximum breadth, when the anterior halves spread apart.

Similar results are obtained by splitting the posterior ends of the worms in two. If the cut extends forward almost to the new head each half produces a pharynx and completes itself on the inner side until each may be as broad as the original worm, Fig. 13 *B*. Where they join the anterior, single part a fold is formed at right angles to the surface of the worm connecting the two halves to the single part. The fold is the result of the halves being wider than half of the anterior, single part. I have kept a number of worms of this sort, and supplied them with an abundance of food in order to see if the old head could be made to grow to twice the normal size, but this never occurred. There is a maximum size beyond which the head will not enlarge; although if it were cut into pieces each piece would make a new worm, and the sum of all the heads of the new worms might be many times greater than that of the maximum head of a single worm. It is only by doubling the structures in the head that a larger head or a number of heads can be made.

If instead of splitting the worm along the middle line a piece is partially split from the side, the latter will also make a new body with pharynx, etc. The question arises whether a piece of this kind can be brought to the same size as the larger part by constant feeding, or whether beyond a certain point its growth will be held in check by the size of its connection with the old part. I can not give a final answer to this question as yet, but I think that it would be difficult to make the piece enlarge beyond a certain point. The opportunity that is given in these experiments of feeding independently each new part so that as far as food is concerned the pieces can be forced to grow to their maximum size opens up a number of interesting questions that I shall hope to enter into and discuss more fully at another time.

Fig. 13.



In my former paper[1]) I described several cases in which one of the posterior halves of a bifid worm was cut off. It was found that if the part was cut off by an oblique cut near its juncture with the other part (as shown in my former paper [1900 A] by the line *o—o* in Fig. 26 *B*) a new body was not formed, but the worm completed itself at the cut-side. If one of the posterior parts was cut off further out, i. e., between its region of union and the pharynx a tail-like outgrowth developed, but I did not determine if a new body would be produced. I have since repeated this experiment several times, and have found that a new posterior part develops which after feeding for several months grows almost to the size of the other piece.

---

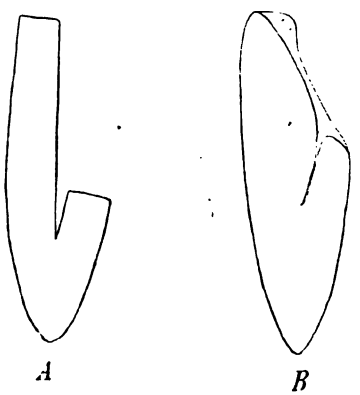
[1]) Page 96 Fig. 6 *B*, line *p—p*.

A number of experiments were made also by cutting off one part of a worm with a double anterior end. It was found if the cut was made anterior to the pharynx, Fig. 12 *C*, *x*—*x*, a new head was produced, but if cut off behind the pharynx, as shown by the line *y*—*y* in Fig. 12 *C*, no new structure appeared. The worm produced a new side and later became symmetrical.

The Inhibiting Effect of an Anterior Cut-Edge on a Posterior Cut-Edge.

In some experiments described in two of my former papers (1900, Fig. X, and 1900 A, Fig. 24) I have pointed out how difficult it is to obtain the development of two heads from cross-cuts made at two levels that are united by a longitudinal cut-edge. The result is due in part to the bending over of the longer side so that the more posterior cut-edge is covered, but this is only a part of the explanation. In one case[1] I found an incomplete structure (whose nature I could not make out) appearing at the lower level. Some new experiments throw new light on these phenomena, and also give, I believe, a deeper insight into one of the internal factors of regeneration.

Fig. 14.



If the anterior end is cut from the worm, the body partially split into two equal parts, and one of the halves is cut off near the bottom of the last cut, as indicated in Fig. 14, there are exposed two cut-ends at different levels. The pieces will fuse along the middle line unless they are for a day or two repeatedly separated. If the pieces fuse along the middle line a single head develops, and this head is always at the anterior cross-cut. New tissue appears along the inner edge of the longer half-piece that is at its most posterior end continuous with the new tissue covering the posterior cross-cut. A new head is not produced by the latter, and this region is slowly changed into the side of the new worm. If, however, the longer and the shorter half-pieces are kept from fusing along the middle line a new head generally develops on the posterior cross-cut. The tendency of the two pieces to unite is very strong, and this may occur even after they have been kept apart for several days. In such cases the new tissue over the posterior cross-cut may have begun to make a head, but its connection with the other side may now interfere with the further development of this head. As a result an outgrowth takes place that has no definite form, and may be mistaken for a tail. It is, however, I think an incomplete head, so far as it is anything. This is shown partly by the way it moves forward, as a head would do, and also by the fact that if the piece is cut off a new tail is produced from its posterior end.

---

[1] (1900) Fig. 24 *C*, *C'*.

The experiment shows that the connection of the shorter piece with the longer one inhibits in some way the development of a head at its cut-edge. This is due, in part, most probably, to the connection of the shorter part with the longer; but more important, I think, is the continuous connection between the anterior and posterior cross-cuts by the new tissue along the inner side of the longer piece. It can not be shown positively that the latter statement is correct, but it is at least made probable by the following case. If the anterior two-thirds of a worm is split in two each part makes a new head and a new inner side, Fig. 12 *C*, if then one piece is cut off near its base a new head seems to appear more often from the cut-surface than in the last experiment. In this case the new tissue at the cut-surface is not connected by new tissue along the inner side of the longer half, but by the tissue that has already differentiated. This connection does not seem to interfere as much with the development of the cut-surface as in the first case, in which the material at the inner edge is new. What kind of influence, we naturally ask, can the anterior tissue that forms a head exert on the new, posterior tissue through the connecting new tissue? It seems to me the results are very similar to those that take place in a piece of a plant, as when a twig is cut from a willow. Only the apical shoots develop, while those at the base of the piece remain inactive. The development of the former inhibits, in some way, the development of the latter, as can be shown by cutting the piece in two near the base when the basal shoots will now begin to develop. That these phenomena are connected with the innermost relations between the parts of the organism there can be, I think, little doubt. We are not able at present, and we may never be able, to account for the changes themselves, but every clue that we can get as to the nature of the action is well worth following up. The way in which I can most clearly picture to myself the kind of action that is taking place in these cases is that some sort of strain must be present that controls the growth. It is as though the anterior part held in check the growth of the posterior part by a pull on the new material of such a sort that it inhibits the growth of the posterior part. The result can not be accounted for, so far as I can see, as the result of the transfer of substances, or of an imaginary action at a distance (Fernkraft), or in any other way that has as yet been proposed. On the other hand the idea that the result is connected with some sort of strain, or tension in the protoplasm gives us not only a new point of view, but enables us more clearly to conceive how the results are brought about. It is as though the more anterior region produces a pull on the more posterior part, and this pull conteracts the growth of the new part. This same condition must be supposed to be present both in the old as well as in the new part, but the experiments seem to indicate that the strain is more strongly exerted through newly forming tissue, than through old parts, and in the new region this condition of strain is the determining factor regulating the growth of the new parts.

A very striking example of how this hypothesis can apply is found in the regeneration of the tail of fishes, especially when an oblique cut has been made. As I have shown in a former paper (1900 B) the new tissue must be supposed to have the same power of growth along the entire length of the oblique cut-edge, but we find that the maximum growth takes place only at the innermost part of the oblique edge, and is checked at all other points beyond this, and proportionately suppressed according to the distance of any point from the region of maximum growth. This holds true for Fundulus that has a rounded tail. A more interesting case is that of Stenopus with a bilobed, or swallow-tail. If the tail is cut off by an oblique cut the new growth is most rapid near that part lying nearest to the base of the tail, where the lower lobe is developing. The growth is least rapid in the middle of the tail, but increases again near the upper edge where the upper lobe is developing. The latter develops less rapidly, hovever, than does the lower lobe. The maximum possible growth takes place only in one region; and in all other regions the growth is held in check, so that the typical form of the tail is assumed. This regulation of the rate of growth at different points can be, I think, most readily conceived as the result of a difference in tension throughout the new parts. My experiments prove, I think, that at any level the growth may be as rapid as at any other, that is, there are no physiological restrictions in any region, except in so far as formative factors inhibit the growth. I can picture to myself much more clearly this balance between the parts as due to a state of relative strain in the different regions than in any other way that has as yet been suggested to account for this and similar phenomena of regeneration. I shall hope to examine more fully in the near future the relation between the action of this supposed strain and the phenomenon of growth, that appears also to be regulated by the same condition of strain. This statement must not be taken in too general a way, for it is evident that the formation of a tail at one end of a lateral edge and a head at the other, or of roots at the lower end of a piece of a plant and shoots at the other end, show that certain kinds of growth take place where the strain is supposed to be greatest. My view is not that a condition of strain inhibits all kinds of growth, but that the relative growth and even the kind of growth of different regions is determined by the relations of strain throughout the part.

Summary.

1) The formation of a heteromorphic head in Planaria lugubris from the posterior end of an anterior piece, Fig. 1, is not due to the cut passing through the brain, or to the absence of a part of the digestive tract in the piece.

2) From a piece cut off just behind the eyes and, therefore, in front of the entire reproductive system it is possible to rear a sexually mature worm, Fig. 2.

3) Cross-pieces of P. lugubris from the anterior end of the body, Fig. 3 *A*, regenerate only a head at the anterior end, but a long posterior part. Cross-pieces from the middle of the worm, Fig. 3 *B*, regenerate an equal amount anteriorly and posteriorly. Cross-pieces from the posterior end, Fig. 3 *C*, regenerate a long anterior end, and very little at the posterior end. In P. lugubris the regeneration takes

place mainly in the new parts, while in P. maculata the old part plays a more important rôle.

4) If regenerating worms are fed the old tissue loses very little in amount, and the new tissue grows faster, Fig. 5 *B—D*. If the worms are kept without food the old tissue loses more and the new part grows less, a smaller worm is formed, Fig. 5 *E—G*.

5) The new head in half cross-pieces, Fig. 6 *Aa*, lies nearer the inner side of the piece, and the inner eye develops later than the outer. The explanation of the latter is due to the smaller amount of development on the regenerating, inner side of the piece.

6) The new head developing from an oblique surface, Figs. 8 and 9, lies on one of the outer sides of the piece, and on that side that lay nearer the old anterior end. Between the median line of the new head and the old median line a connection is established by obliquely lying, median organs as shown in Figs. 9 *b* and *f*.

7) The formation of one, or of two heads in the anterior angle of a piece split almost in two (except at the anterior end), and the formation of a half-head by each half, if the cut extends into, and nearly through the old head find a common explanation as due to the formation of new structures at the anterior end of the new material at the side. The new heads do not appear to be heteromorphic formations.

8) If the anterior end is split in two, so that two heads are formed these heads are much smaller than the original heads, especially if the division between them does not extend far posteriorly, Fig. 12 *A*, *B*. If the anterior end is split further posteriorly, Fig. 12 *C*, the new heads grow to larger size, and may even become nearly full-sized. The explanation of this difference is due to the area of union of the heads to the body, restraining the enlargement of the new head, and its influence is the greater in proportion to its nearness to the new heads. If the anterior end is split into unequal parts the new heads are in proportion to the sizes of the parts on which they form, and their subsequent growth is determined by the area of the uniting region and its distance from the new heads.

9) If a worm has an anterior and a posterior half cut-surface, as shown in Figs. 14 *A*, a head develops only on the anterior surface, if the two surfaces are connected by a line of new tissue along the inner side of the longer half, Fig. 14 *B*. But if the longer and the shorter halves are kept apart two heads often develop. It is suggested that the influence of the anterior tissue on the posterior is brought

about by a tension that restrains the development of the posterior head, and the same explanation is extended to other phenomena of regeneration.

Zusammenfassung.

1) Die Bildung eines heteromorphen Kopfes bei Planaria lugubris am hinteren Ende eines Vorderstückes (Fig. 1) ist nicht vom Durchgang des Schnittes durch das Gehirn verursacht, auch nicht durch die Abwesenheit eines Theils des Verdauungstractus in dem Stück.

2) Es ist möglich, von einem Stück einen geschlechtsreifen Wurm zu erhalten, welches gerade hinter den Augen und somit vor dem gesammten Fortpflanzungssystem abgeschnitten wurde (Fig. 2).

3) Querabschnitte von Planaria lugubris vom vorderen Körperende (Fig. 3 *A*) bilden lediglich an der vorderen Seite einen (kurzen) Kopf, aber ein langes Hintertheil. Ebensolche aus der Wurmmitte (Fig. 3 *B*) regeneriren vorn und hinten etwa gleich viel. Querabschnitte vom Hintertheil (Fig. 3 *C*) regeneriren ein langes Vorderende und nur sehr wenig nach hinten zu. Bei P. lugubris finden die Regenerationsvorgänge lediglich in der Neubildung statt, während bei P. maculata das alte Gewebe eine wesentlichere Rolle spielt.

4) Wenn man die Würmer während der Regeneration füttert, so wird die Masse des alten Gewebes dabei nur sehr wenig angegriffen und das neue Gewebe nimmt rascher zu (Fig. 5 *B—D*). Lässt man sie hungern, so verliert das alte Gewebe mehr Masse, die Neubildung wächst langsamer und man erhält einen kleineren Wurm (Fig. 5 *E—G*).

5) Der neue Kopf bei längshalbirten Querabschnitten (Fig. 6 *Aa*) liegt näher der Medianseite des Stückes und das median gelegene Auge entwickelt sich später als das andere. Die Erklärung für das Letztere liegt in der langsameren Entwickelung bei der Regeneration der Medianseite des Stückes.

6) Der neue Kopf, der sich an einer schiefen Oberfläche entwickelt (Fig. 8 und 9), liegt auf einer der Außenseiten des Stückes und zwar auf der dem alten Vorderende zunächst gelegenen. Zwischen der alten Mittellinie und der des neuen Kopfes besteht ein Zusammenhang durch die Schräglagerung der medianen Organe (vgl. Fig. 9 *b* und *f*).

7) Die Bildung eines oder zweier Köpfe im vorderen Winkel eines größtentheils (mit Ausnahme des vorderen Endes) längsgespaltenen Stückes und die Bildung eines halben Kopfes seitens jeder Hälfte, wenn der Schnitt sich in und fast durch den alten Kopf erstreckt, findet eine einfache Erklärung als veranlasst durch die Entstehung von Neubildung am vorderen Ende des Neumaterials an der Seite. Die neuen Köpfe scheinen keine heteromorphen Bildungen zu sein.

8) Spaltet man das vordere Ende und veranlasst so die Bildung von zwei Köpfen, so sind diese viel kleiner als der ursprüngliche Kopf, besonders wenn die Theilung zwischen ihnen sich nicht weit nach hinten erstreckt (Fig. 12 *A*, *B*). Wird der Schnitt nach hinten verlängert (Fig. 12 *C*), so wachsen die beiden Häupter zu bedeutenderer Größe, ja selbst zur normalen. Die Erklärung dieses Unterschiedes hängt ab erstens von der Breite des Stückes, zweitens von der Entfernung des regenerirenden Kopfes von dem gemeinsamen Körper. Spaltet man das Vordertheil längsweise in einen breiten und einen schmalen

Streifen, so entspricht die Größe der neuen Köpfe den vorhin angegebenen Umständen.

9) Hat ein Wurm eine halbdurchschnittene vordere und hintere Oberfläche (vgl. Fig. 14 *A*), so entwickelt sich ein Kopf nur an der vorderen der beiden Querflächen, wenn sie durch einen Streifen neuen Gewebes entlang der Innenseite der größeren Hälfte verbunden sind (Fig. 14 *B*). Bleiben aber größere und kleinere Hälfte getrennt, so entwickeln sich oft zwei Köpfe. Es wird angenommen, dass der Einfluss des vorderen Gewebes auf das hintere zu Stande kommt durch eine Spannung, welche die Entwickelung des hinteren Kopfes hemmt, und dieselbe Erklärung wird auf andere Regenerationserscheinungen ausgedehnt.

References.

Bardeen, C. L., On the Physiology of Planaria maculata with especial reference to the Phenomena of Regeneration. The American Journal of Physiology. V. 1901.

Dalyell, J. G., Observations on Some Interesting Phenomena in Animal Physiology Exhibited by Several Species of Planariae. Edinburgh 1814.

Duges, A., Recherches sur l'Organisation et les leveurs des Planariées. Ann. d. Sc. Nat. XV. 1828.

Van Duyne, J., Über Heteromorphose bei Planarien. Archiv f. ges. Physiol. LXIV. 1896.

Faraday, M., On the Planariae. Edinb. New Phil. Journ. XIV. 1833.

Flexner, S., The Regeneration of the Nervous System of Planaria larva and the Anatomy of the Nervous System of Double Headed Forms. Journal of Morphology. XIV. 1898.

Johnson, J. R., Observations on the Genus Planaria. Phil. Trans. Royal Soc. London. 1822.

—— Further Observations on Planariae. Ibid. 1825.

Lemon, C. C., Notes on the Physiology of Regeneration of Parts of Planaria maculata. Biolog. Bulletin. I. 1900.

Lillie and Knowlton, On the Effect of Temperature on the Development of Animals. Zool. Bulletin. I. 1897.

Morgan, T. H., Experimental Studies of the Regeneration of Planaria maculata. Archiv f. Entwickelungsmech. VII. 1898.

—— Regeneration: Old and New Interpretations. Biological Lectures from the Marine Biological Laboratory of Woods Holl. 1899.

—— Regeneration in Bipalium. Archiv f. Entwickelungsmech. IX. 1900.

—— Regeneration in Planarians. Ibid. X. 1900 A.

—— Regeneration in Teleosts. Ibid. X. 1900 B.

Randolph, H., Observations and Experiments on Regeneration in Planarians Ibid. V. 1897.

Voigt, W., Künstlich hervorgerufene Neubildung von Körpertheilen bei Strudelwürmern. Sitzungsber. d. niederrhein. Gesellsch. f. Natur- u. Heilkunde zu Bonn. 1899.

Wyman, J., An Account of Some Experiments on Planaria Showing their Power of Repairing Injuries. Proc. Boston Soc. Nat. Hist. IX. 1865.

Regeneration in Tubularia mesembryanthemum.

By

N. M. Stevens.

With Plate XVIII and 1 figure in text.

Eingegangen am 16. August 1901.

The material for this work was obtained at the Naples Zoological Station in June and July, 1900, by Prof. T. H. **Morgan**, in connection with his study of regeneration in Tubularia.

The phenomena of regeneration in Tubularia, as observed in the living animals, have been fully described by **Bickford** ('94), **Driesch** ('96, '97, '99) and **Morgan** ('01). The chief object of this paper is to give the histological evidence 1) in regard to the method of formation of the new hydranth, 2) in regard to the distribution and function of the red granules, — the so-called red formative substance of **Loeb** ('92) and of **Driesch** ('99).

Technique. — The material, consisting of long and of short pieces in various stages of regeneration, was fixed in corrosive acetic, — saturated corrosive sublimate with 5 % glacial acetic acid. The pieces were embedded in paraffine in the usual manner, and sections cut about 5 μ thick, both transversely and longitudinally. After experimenting with **Heidenhain**'s iron-haematoxylin and **Delafield**'s haematoxylin in combination with various plasma stains, it was found that the most satisfactory results were obtained by staining on the slide with **Delafield**'s haematoxylin, running up to 100 % alcohol, and then treating with a solution of picric acid in 100 % alcohol, clearing in xylol and mounting in balsam. By this method the chromatin in dividing cells is stained nearly black, the endoderm granules yellow, the cytoplasm a pale grey, and the glandular endoderm of the hypostome purple.

Regeneration. — In the earliest stages of regeneration, after the closing in of the cut ends, as described by **Morgan** ('01), the sections show three processes to be going on simultaneously, — thinning out of the distal end, cell division in the part of the coenosarc which is to form the new hydranth, and breaking down of the endodermal ridges and of the cross bridges between them in the hydranth region.

Thin distal end. — When thickening of the coenosarc for the formation of a new hydranth is first discernible, in pieces not exceptionally short, the thinner distal end extends through from 10 to 15 sections; a few hours later when the folding of the tentacle anlagen begins, it extends through 25 to 30, or in some cases, more sections. As there is no evidence of migration of cells from this region, and the extension forward observable in the living animal is not sufficient to account for the phenomenon, we are forced to the conclusion that the increasing thinness of the distal end is due to slight withdrawal or contraction in a proximal direction of the coenosarc of the hydranth region.

An average section of the stalk outside of the region of regeneration, and where no contraction away from the wall has occurred is shown in Fig. 1, a similar section of stalk slightly contracted in Fig. 2; a very thin end in Fig. 3, all being drawn to the same scale. Figs. 12 and 13 are from distal and proximal ends of the same piece, showing the relative thickness of the coenosarc in different parts after considerable thickening has occurred in the hydranth region, the distal end being much thinner and passing gradually into the thicker region where rapid cell division is going on and the cells are changing from the cubical or flattened form of the stalk to the columnar form. Figs. 4 and 5 show cell division in both ectoderm and endoderm together with breaking down of the endodermal ridges: Fig. 4 was taken from a long piece in which the ridges were very small, Fig. 5 from a short piece in which they were unusually large. In the sections from which Fig. 5 was taken, the lumen of the whole piece was filled with loose cells, separate granules, and groups of granules held together by cytoplasm.

In two portions of the thickened part of the coenosarc longitudinal folds appear, forming the tentacle anlagen, and from this time on most of the dividing cells are found in the developing tentacles.

The method of formation of the proximal circle of tentacles is shown in Figs. 6—9, — a gradual folding of the coenosarc longitudinally, and a final pinching off of the tentacles from the body of the hydranth, the increase in surface of ectoderm being due partly to cell division, partly to change in the cells from a more to a less columnar form. These figures were taken from the same series of sections, the process of folding being always more advanced at the distal than at the proximal end of the anlage.

The distal tentacles of some long pieces are formed in exactly the same manner, but in most cases the method is somewhat different, as shown in Figs. 10 and 11: a rod of endoderm is folded off, and the ectoderm gradually grows around this to form the tentacle, the glandular endoderm of the hypostome being differentiated at the same time.

As the hydranth approaches maturity, it pushes forward, growth in all parts still taking place by cell division, the thin end contracts becoming a part of the hypostome, and opens distally to form the mouth.

Cell Division. — Mitosis is more abundant and continues longer in the ectoderm than in any part of the endoderm except the glandular hypostome region. The chromosomes are small, very numerous and densely crowded together in all phases.

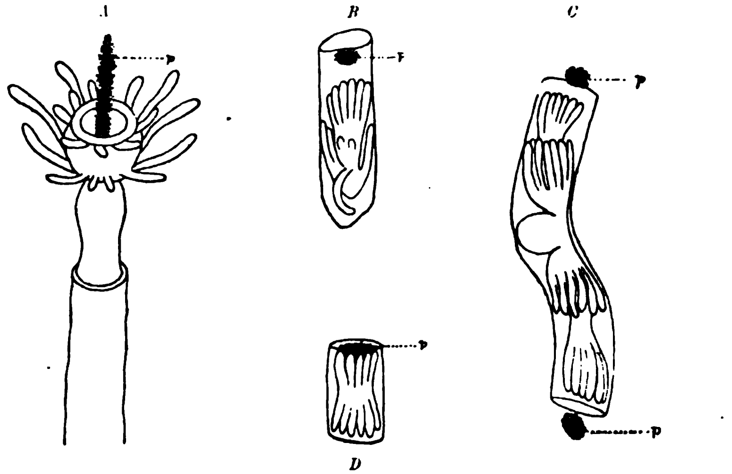
During the thickening of the hydranth region, before folding begins, the spindles lie parallel to the surface of the tube and in various positions; but most of them are oriented either transversely or longitudinally and the number of spindles lying in these two directions is approximately equal.

Granules. — In the sections from this preserved material, a large number of translucent granules, varying in size from less than 1 μ to 8.5 μ in diameter were observed in the endoderm of both adult and regenerating specimens (Figs. 4 and 5 *g*). These granules stain various shades of yellow and orange with picric acid. They are very abundant in the ridges of the old stalk, in the endoderm of the tentacle anlagen and in the endoderm of the newly formed tentacles, while in the thin end and in the hypostome region they are very rare (Figs. 3 and 6—11). They are also abundant in the lumen of sections that show disintegration of the ridges, especially in short pieces with large ridges (Fig. 5).

It was thought at first that these might be the red granules described by the various authors. Fortunately I was able to obtain at Woods Holl, Mass., a nearly related species Tubularia crocea (Parypha crocea). The endoderm granules were identified both in living material, and in sections from pieces fixed and stained like the Naples material. In the living tissue, they were translucent, colorless, or slightly yellowish, and identical in form, size and distribution with the yellow granules of the sections of Tubularia mesembryanthemum.

In the same cells and also in other parts of the endoderm were found the irregular grains and masses of red pigment, which in the Naples material had been dissolved out by the alcohol. Pieces fixed in corrosive acetic and embedded as quickly as possible showed some of the pigment remaining in parts of the hydranth where it was most abundant, but it had all disappeared in stalk and tentacles where the granules were scattered.

Figs. A—D.



Regenerating Tubularia crocea extruding waste pigment derived from disintegration of endodermal ridges.

Observation of regenerating pieces of Tubularia crocea showed that the red particles seen in the circulation of this form, — and doubtless the same is true for the Naples species, — come with the other debris with which the circulation is filled a few hours after cutting, from the disintegrating ridges. Pieces were opened and the circulating material examined under a sufficiently high power to identify nuclei, translucent endoderm granules, and red pigment granules.

Moreover, in this species, the newly formed hydranth, very soon after emerging from the tube, disgorges one or more large masses of red pigment granules, practically free from other cell material.

In short pieces the pigment mass is thrown out within the coenosarc (Figs. *B* and *C*). Except for these pigment masses, the circulating fluid is at this time clear and free from red granules. The indication is that the red granules, far from being »formative substance«, are in fact the only part of the cell material from the disintegrated endodermal ridges, which the animal cannot make use of in regenerating a hydranth, and therefore rejects at the first opportunity.

Conclusions.

1) In regeneration of Tubularia mesembryanthemum, cell division in both ectoderm and endoderm is an important factor in producing the increased amount of tissue required to form a hydranth.

2) Cell division here occurs in the ordinary large endoderm and ectoderm cells, — not in interstitial cells nor in any specialized germ cells.

3) The region of the old stalk thickened by growth accompanied by cell multiplication, is remodeled into the hydranth form, cell-division going on during the process of transformation.

4) The red granules seen in the circulation of regenerating pieces of Tubularia are derived from the disintegrating endodermal ridges, and are ejected by the young hydranth soon after it emerges from the tube. They are waste material rather than »formative substance«.

Woods Holl, Mass., Aug. 1901.

Zusammenfassung.

1) Bei der Regeneration von Tubularia mesembryanthemum sind Zelltheilungen sowohl im Ektoderm wie im Entoderm ein wichtiger Faktor bei der Hervorbringung des Gewebszuwachses, der zur Bildung eines neuen Hydranten erforderlich ist.

2) Man begegnet der Zelltheilung dabei in den großen Entoderm- und Ektodermzellen, nicht in den interstitiellen Zellen oder irgend welchen specialisirten Keimzellen.

3) Der ursprüngliche Stielbezirk, verdickt durch Wachsthumsvorgänge mit Zellvermehrung, wird zur Hydrant-Gestalt umgebildet, indem die Zelltheilungen während des Umbildungsprocesses andauern.

4) Die bei der Cirkulation in den in Regeneration begriffenen Stücken beobachteten rothen Körnchen stammen von dem Zerfall der endodermalen Bestandtheile her und werden von dem jungen Hydranten bald nach dessen Auftauchen aus der Röhre ausgeworfen. Sie sind viel mehr überflüssiges unorganisirtes Material als Bildungssubstanz.

Since this paper was written, I have been able to study Tubularia mesembryanthemum at Naples, and have again observed the ejection of red pigment granules (Figs. *A—D*). The phenomenon is not so striking in this species, because the ridges are smaller and fewer in number; the amount of pigment thrown into the circulating fluid from the disintegrating ridges is therefore so much less that the phenomenon might easily elude observation.

Naples, Oct. 26, 1901.

Literature.

Bickford, Elisabeth E., '94, Notes on Regeneration and Heteromorphosis of Tubularian Hydroids. Journ. of Morph. IX. 1894.

Driesch, H., '96, Zur Analyse der Reparationsbedingungen bei Tubularia. Vierteljahrsschr. Nat. Ges. Zürich. 16. 1896.

—— Studien über das Regulationsvermögen der Organismen.

'97. I. Von den regulativen Wachsthums- und Differenzirungsfähigkeiten der Tubularia. Vierteljahrsschr. Nat. Ges. Zürich. 5. 1897.

'99. II. Quantitative Regulation bei der Reparation der Tubularia. Archiv f. Entwickelungsmech. IX. 1899.

Loeb, J., '92, Untersuchung zur physiologischen Morphologie der Thiere. II. Organbildung und Wachsthum. Würzburg 1892.

Morgan, T. H., '01. Regeneration in Tubularia. Archiv f. Entwickelungsmech. XI. 2. 1901.

Description of Figures.

Plate XVIII.

Magnification: Figs. 1—11 Zeiss D. oc. 8, Cam.
Figs. 12—13 - A. - 8, -

ec ectoderm, *en* endoderm, *g* endoderm granules.

Fig. 1. Section from stalk 2—3 mm proximal to tentacle anlagen, not contracted away from the perisarc.

Fig. 2. Similar section slightly contracted.

Fig. 3. Section from thin distal end.

Fig. 4. Section from hydranth end of a long piece with small ridges breaking down.

Fig. 5. Section from a short piece with large ridges, showing cell division and disintegration of ridges.

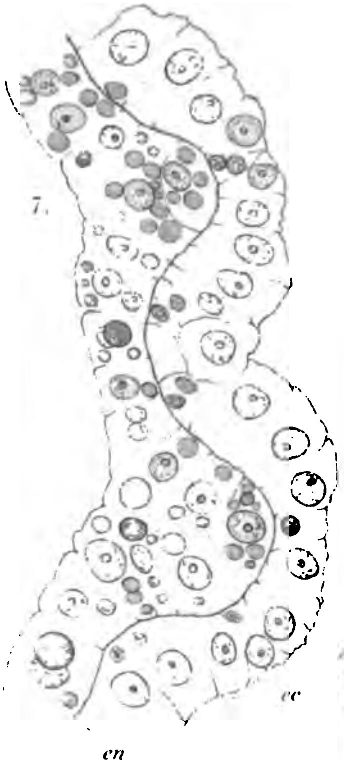
Figs. 6—9. Sections from proximal tentacle anlage, showing successive stages in the process of tentacle formation.

Figs. 10—11. Sections from distal tentacle anlage, showing simultaneous folding off of tentacle endoderm, and differentiation of the glandular endoderm of the hypostome.

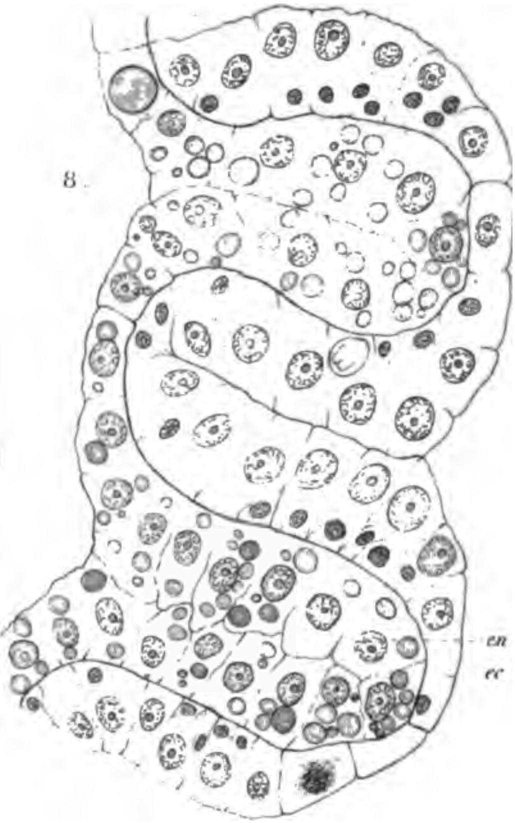
Fig. 12. Longitudinal section, showing relation of thin end to the thickening hydranth region.

Fig. 13. Proximal end of same piece.

7.

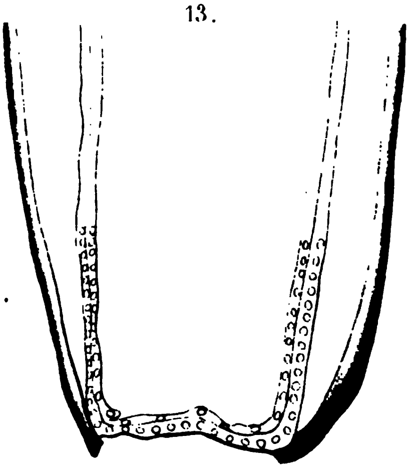


8.



12.

13.



Notes on Regeneration in Planaria lugubris.

By

N. M. Stevens.

With Plate XVII and 2 figures in text.

Eingegangen am 16. August 1901.

Planaria lugubris, a species of planarian found in considerable abundance in the streams about Bryn Mawr, has been the subject of many of Prof. T. H. MORGAN's experiments in regeneration. Comparing the results of such experiments on this form and on Planaria maculata, he observed that the two species were somewhat different in their method of regeneration. It was therefore thought to be advisable to investigate the histological side of the question, in order to determine, if possible, whether the new tissue which appears at the cut surface in much greater amount in P. lugubris than in P. maculata, results from migration of cells from the old part, or from cell multiplication at the cut surface and later in the new part, or from both processes. The differentiation of tissues and organs out of the new material will also be considered.

The planarians were collected from partially submerged green plants and from the undersides of dead leaves along the margins, of rapidly, running streams in October and November, 1900, and again on the undersides of flat stones in the same streams in April and May, 1901. During the winter they were kept in covered crystallization dishes, in spring water which was changed every two days. They were fed on pieces of earthworm which they ate ravenously, and seemed to thrive upon, reaching their maximum size and holding it as long as fed.

Technique. — The planarians were cut in various planes; the pieces were kept in spring water, which was changed daily, and were fixed at intervals of a few hours in corrosive acetic, — saturated corrosive sublimate and 3—5 % glacial acetic acid. After the usual processes of hardening in alcohols, embedding in paraffine, and sectioning, the material was stained with DELAFIELD's haematoxylin, and differentiated with orange. Other combinations of stains were tried, but this method gave the best differentiation of both old and new tissue.

All nuclei, with the exception of those of the ova, are stained blue or purple; the spermatozoa and the chromatin of dividing cells are a deep blue and very sharply defined; the mucus and the cytoplasm of the ova are also blue. The rhabdites are black or sometimes dark brown. Muscle fibres, fat cells, eosinophile leucocytes, endoderm cells of the pharynx, and granules of the endoderm cells of the alimentary tract and of the yolk cells are stained yellow or orange. Nucleoli of the ova, endoderm and yolk nuclei are also yellow.

Method. — Though most of the work was done on microscopical sections, observations were also made on the living material, with measurements and drawings to scale. Two series of such drawings, showing changes in form — »morphallaxis« (MORGAN) — appearance and location of eyes and pharynx, are reproduced in Figs. *A*, *a—h*, a piece from the region between the neck and pharynx, and *B*, *a—h*, a tail piece cut just posterior to the genital pore. These pieces were taken from a worm that had been kept in the laboratory about five months, and though it was apparently healthy, regeneration was slower than in worms fresh form the stream where they were collected. The drawings were made at intervals of two or three days during a period of nineteen days. The general changes in form have been described and figured by MORGAN ('00). It will be noticed in comparing the two series that changes in form, as well as development of eyes and pharynx are more rapid in the prepharynx piece than in the tail piece. The change in form of the head from *Ad* to *Ae*, and from *Bf* to *Bg* should be noted; also the projection of the old part forward between the eyes and backward or forward on either side of the pharynx (Fig. *A*, *d—h*, Fig. *B*, *e—h*): these points will be discussed later in connection with the sections.

Ectoderm. — For microscopical study of the process of closing in of the cut end, material was fixed at intervals of two hours after cutting.

In some cases the wound is closed by immediate contraction

Fig. *A.*

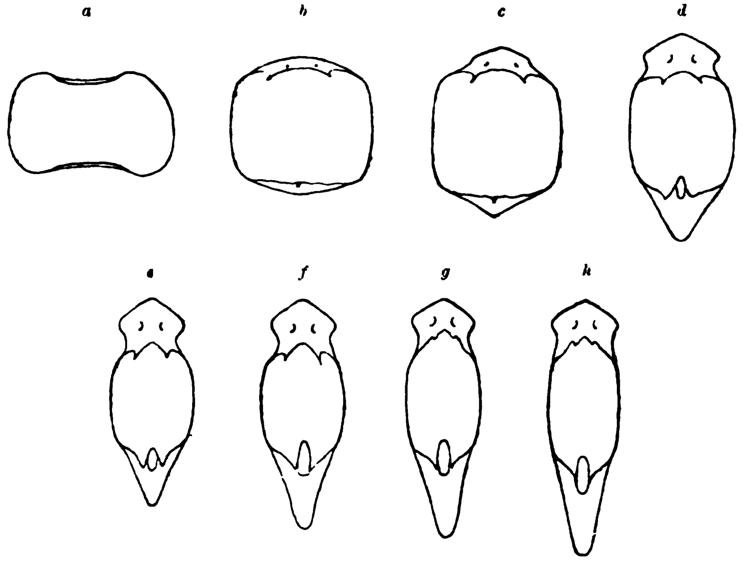


Fig. *B.*

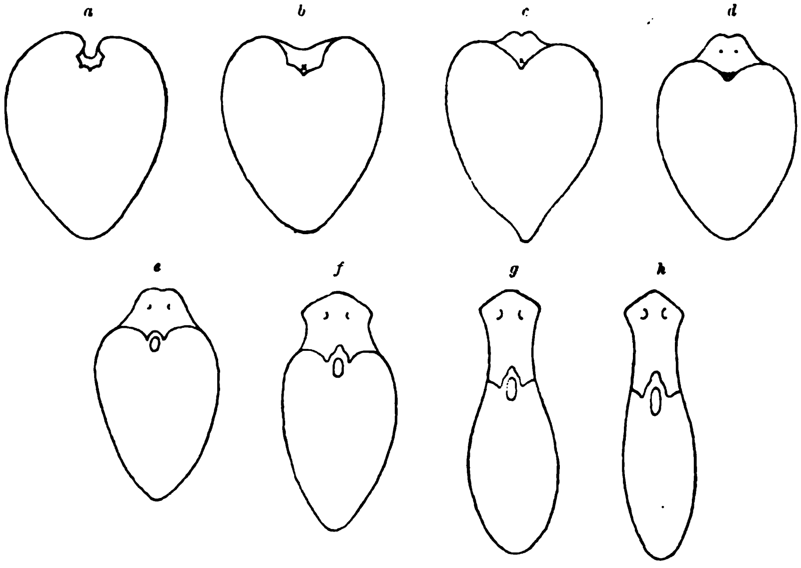


Fig. *A.* Piece cut anterior to the pharynx on Apr. 27, from a specimen which had been kept about five months in the laboratory. × pharynx anlage.

Fig. *B.* Tail piece from same worm cut just posterior to genital pore.

The figures in both cases are from freehand drawings to scale, length and breadth having been measured by watching the animal crawl over a millimeter scale placed under the glass dish.

a	2 days after cutting	*e*	11 days after cutting.
b	4 - - -	*f*	13 - - -
c	6 - - -	*g*	16 - - -
d	8 - - -	*h*	19 - - -

of the surrounding muscles, and subsequently by overgrowth of ectoderm, without loss of tissue; but in most cases there is to be observed in both living material and in the sections an extension and sloughing off of considerable material from the cut end during the first hour after cutting and of some cells later during the relaxation of the muscles and overgrowth of the ectoderm. The new covering of ectoderm is frequently burst open in fixation or broken in sectioning, but it is usually not difficult to distinguish such breaks from cases of incomplete closing.

The time required for complete closing over of the end by ectoderm cells varies greatly without apparent cause: some sections show perfect closure after ten hours, others not after twenty-four hours. Fig. 1 *a*, Pl. XVII shows the appearance of a longitudinal dorso-ventral section of a piece anterior to the pharynx, sixteen hours after cutting. The covering of the cut surface is effected not by multiplication of ectoderm cells, nor by differentiation of new ectoderm cells from internal embryonic tissue, but by elongation and forward movement of the old ectoderm cells on the margin of the wound, the greater part of the surface being covered by the longer cells from the dorsal side. Fig. 1 *c* shows approaching ectoderm cells in another section of the same piece.

Every section mounted during the year has been carefully examined for karyokinesis in the ectoderm of the new part, but no undoubted case of it has been discovered. Fig. 1 *b* shows a peculiar aggregation of nuclei common in ectoderm that has recently closed in. Possibly we may have here direct division of the nuclei, but there is no certain evidence that such is the case. Boundary lines between the ectoderm cells in the stage shown in Fig. 3 are difficult to make out, but the ectoderm is perfectly distinct in outline and staining qualities from the other cells. The cells, as they move forward always have a thin layer of pigment on their outer border and occasionally a rhabdite is carried along (Fig. 1 *a—c*).

By the fourth or fifth day, the new ectoderm, at first extremely thin and appearing more like a syncytium than a layer of flat cells, assumes its usual appearance, — cubical or columnar cells filled with rhabdites. There is no evidence of further migration of old ectoderm cells after the first closing in, nor has cell-division been observed in either regenerating ectoderm, or embryonic ectoderm; but in both, many cells containing rhabditis appear to be migrating into the ectoderm.

Parenchyma, Undifferentiated Tissue. — Scattered among the muscle fibres, gland cells and excretory tubes, between the digestive tract and the external layers of muscle of the mature planarian are cells of the type shown in Fig. 2 *a—d.* These cells have finely granular bodies and rather small, deeply staining nuclei. Some have longer processes and closely resemble stellate or embryonic connective tissue cells of higher animals; others have the appearance of wandering cells, or leucocytes. These cells are rarely found dividing in the normal adult worm, but in the pieces, perhaps at the stimulus given by the injury, they begin to multiply even before the ectoderm has completely closed over the cut. By the time that the ectoderm covering is complete there is a well defined layer of these undifferentiated cells, many of them in various stages of karyokinesis, between the new ectoderm and the old internal tissue (Fig. 1*a*).

The amount of this embryonic tissue increases rapidly by division of its own cells and also probably to some extent by migration of similar cells from the old tissue; for a great many of the embryonic or parenchyma cells described above, are dividing in the old tissue near the wound and even throughout short pieces cut at both ends, without any apparent increase in the number of such cells in the region. Eosinophile leucocytes, probably migrants from the old part, are occasionally seen among the embryonic cells of the new part.

Differentiation of new tissue. — After about forty hours, in cases of very rapid regeneration, and in most cases during the third day, differentiation of embryonic cells into muscle cells, rhabdite and mucus secreting cells, and endoderm cells may be observed; also the penetration of nerve fibres and of branches of the digestive tract from the old into the new part, and the beginning of a new pharynx.

Development of muscle cells is shown in Fig. 3. Longitudinal striation is much more conspicuous in the early stages of differentiation than later, when the fibres appear nearly homogeneous.

Mucous cells are easily distinguished by the purplish brown staining of the cytoplasm, and larger nucleoli (Fig. 4).

Endoderm cells have the granular appearance of embryonic cells, but the granules are larger, more irregular and stain with the orange: they also have conspicuous nucleoli.

Rhabdite secreting cells are chiefly conspicuous for the presence of the deeply staining elliptical rhabdites. The cytoplasm of these cells is more transparent — free from granules — than is that of the other cells, and both body and nucleus of the cell are irregular in outline.

Pharynx. — The pharynx in this species always appears in the new tissue next to the old part as is indicated by the crosses in Fig. *A b, c* and *B b, c.* When the location of a new pharynx is first distinguishable in sections, — from the second to the fourth day, according to the part of the animal from which the piece is taken, and the condition of the worm, — it is merely a part of the embryonic mass, where cells are somewhat smaller and more closely packed together than elsewhere. At this stage dividing cells may be observed in the pharynx region, and in both prepharynx and tail pieces the digestive tract is advancing along the right and left sides, to unite in front of the pharynx in tail pieces and form an axial gut: evidently in this case the presence of an axial gut does not determine the location of the pharynx, as BARDEEN ('01) thinks that it does in every case in P. maculata. After a time cell division ceases in the region of the new pharynx and differentiation of epithelium begins near the centre of the mass of cells. These epithelial cells can be distinguished by their increased size and by the yellow staining of their cytoplasm. This differentiation proceeds forward and backward and results in the axial cavity of the pharynx. Meanwhile a break appears in the posterior part of the region to form the beginning of the pharynx chamber, which gradually extends forward around the developing pharynx. The embryonic cells on the surface of the chamber develop into the characteristic lining cells of different parts.

Fig. 6 shows the new pharynx in a horizontal section from a prepharynx piece, killed after five and a half days. Here the axial opening of the pharynx has made connection with the axial gut of the old part, the pharynx chamber is partly formed, but the cells are only slightly differentiated from the embryonic condition.

Fig. 7 is from a longitudinal, dorso-ventral tail piece 5 days after cutting, and shows a similar stage with the mouth opening already indicated. Connection of the pharynx with the alimentary tract appears in another section.

From this point on, it is simply a question of further differentiation of some of the embryonic cells into the epithelium cells characteristic of the different parts of the pharynx and its chamber; of others into muscle cells, gland cells and nerve cells, and finally the formation of a mouth-opening, by inturning of surface ectoderm.

Fig. 8 shows a case where a small portion of the old pharynx was accidentally left on the piece. The line *x—x* shows the division line between old and new tissue as indicated by the different staining qualities of old and new pharynx tissue. In this and other similar cases we find a portion of the old pharynx chamber left when the end closed in. The piece was fixed after forty-six hours and the new part of the pharynx is much farther advanced than in any case where the new organ is not formed in connection with a part of the old.

In several cases a part of the genital region was included in a tail piece, the cut having been made through the genital pore or just in front of it. In such cases the appearance of the pharynx is delayed, but finally comes in on the anterior margin of the portion of the genital cavity included in the piece (Fig. 11).

In pieces containing the old pharynx uninjured, no new pharynx is formed, but the old pharynx remains functional.

Digestive tract. — The first advance of the digestive tract from the old into the new part of the regenerating planarian seems to be a mass movement, in posterior pieces, of two branches on either side of the region where the new pharynx is forming and in anterior pieces of the axial gut. With the branches of the digestive tract goes a considerable amount of the adjacent tissue especially on the dorsal side where the ectoderm also advances. This was first noticed in such pieces as are figured in Fig. *A*, where the old part is represented as extending backward on either side of the pharynx (Fig. *A*, *d—h*). It was also observed that there was a similar extension forward between the eyes, and that these projections were confined to the dorsal side. The accuracy of this observation was then confirmed by the examination of corresponding sections. Fig. 6 shows a stage similar to Fig. *A d* but from more rapidly regenerating material. The dotted line *x—x*, *x—x* show the division between old and new tissue. Similar figures could be drawn from other pieces.

This pushing forward of the old digestive tract is however only a beginning of the process by which the new region is supplied with its alimentary branches. It is impossible to state positively the manner in which this is effected. The one point that is certain is that the new branches bud out from the old tract making use of the reserve cells which lie at the base of the active endoderm cells. Occasionally one of these reserve cells is found in karyokinesis, but such cases are too rare to account for the rapid growth of the new branches. Cell division is, however, abundant in the embryonic cells around these growing branches and among these cells are found some such as are shown in Fig. 5 *a*. These I have called endoderm cells for they are exactly like those of the ends of the new branches, — cytoplasm granular and staining with orange nucleus with a conspicuous nucleolus. The only explanation that I see at present for the growth of the digestive branches without multiplication of their own cells, is that endoderm cells are differentiated in the embryonic area, and are applied to the branches which originate in the old digestive tract.

Excretory System. — As is well known, the excretory or nephridial system of these planarians consists of a complicated system of branching tubes penetrating all the loose tissue between the digestive system and the ectoderm, but more abundant on the dorsal side and there opening to the exterior between the ectoderm cells, the tubes extending through the basement membrane and between the surface cells nearly to their distal ends.

In sections, the boundaries between the cells that compose these tubes is not evident.

In sections taken from pieces from three to five days old, one of these tubes is found extending into the new part at the head end just dorsal to the digestive tract. Figs. 10 *a* and *b* show cross sections of such, and *c* a longitudinal section from a piece 5 days old: the dotted lines indicate approximately the boundary between old and new parts, the branching end being in the new part. Thus the regeneration of the excretory system, like that of the digestive takes place in connection with the old tubes, but how the sections do not show. Karyokinesis has not been observed in the nuclei of the tubes, however, making it seem probable that cells are added from the embryonic region.

Nervous System. — The nervous system also regenerates in connection with the part left in the original piece, as described by FLEXNER ('98) for P. torvo (maculata). The nerve tracts extend into the new part and a large number of embryonic cells group themselves around these advancing tracts, and become the nerve cells of the new part. Here again, no cell division has been observed in the nerve cells of the old nerve tract. In ordinary sections it is almost impossible to distinguish nerve cells from parenchyma or undifferentiated cells unless their fibres can be traced.

The only difference in the appearance of the nuclei in my preparations is a slightly brownish shade while the nuclei of the embryonic cells before differentiation are decidedly blue. The nerve fibres are more easily traced in specimens that have not been fed for several weeks before cutting: the other tissues degenerate somewhat while the nervous tissue does not to nearly the same extent. A specimen starved for 18 weeks showed head ganglia nearly as large as those in corresponding specimens fed during the same time, while the other tissues were much reduced; everything in the shape of fat or yolk material having entirely disappeared, and the actual size of the animal having been reduced from 11 × 3 mm to 3 × $^3/_4$ mm.

Fig. 7 shows the grouping of undifferentiated cells around the fibre region of a ganglion, developing in a 5-day tail piece: other sections of the series show the connection with the old nerve tract.

Many of the nerve cells appear to be unipolar: the process can be traced only a short distance in the nerve tract. Occasionally a distinctly bipolar cell is observed (Fig. 11) with processes running lengthwise in the lateral cords.

The head ganglia, or brain, and the connecting commissure are usually well developed in 6 or 7-day pieces, and by the 9th or 10th day connections with ectoderm, eyes and lateral sense organs are completely formed. The time of regeneration varies so much that it is impossible to specify exactly, but the regeneration of the various parts runs along in parallel lines. Certain stages of development of pharynx, eyes and brain always appear together (Figs. 6 and 7).

Eyes. — The eyes are first seen in sections from the third to the fifth day, earlier in anterior than in posterior pieces. The earliest trace of them that I have found was in a 3-day prepharynx piece; here each eye was represented by three cells containing a few of the characteristic coarse brown pigment grains, large nuclei and fibrillar cytoplasm.

These first eye cells are very deeply placed in the embryonic material, and judging from the position of older pieces killed from day to day, more cells must be added on the dorsal side of the group. Fig. 12 shows an eye from a piece 6 days old. There is no evidence that these eye-forming cells sink in from the ectoderm, and the fact that the eye is first apparent as a small group of deeply placed cells which is gradually added to, indicates that, as in the case of all the other tissue except possibly the ectoderm, they arise from differentiation of some of the embryonic cells.

Auricular Sense organs. — After the brain is well developed in the new part, there are differentiated in the region of the auricular appendages a series of sense organs which are connected with the brain, each by a conspicuous nerve cord. Each of these organs, as seen in section, consists of a group of from three to five cells shorter and broader than the surrounding epithelium (Fig. 13). They contain no rhabdites and only a little pigment at the distal end. The nuclei are somewhat larger and spherical, while the nuclei of the surrounding ectoderm cells are oval. The cells have longer and more numerous cilia than the other ectoderm cells, are more richly enervated and are connected by a large nerve cord with the brain. These organs, visible with a hand lens in mature worms, are probably organs of smell or taste.

Reproductive System. — A careful study of the regeneration of the reproductive organs has not yet been made, but the work is now in progress. The tail pieces of this species, cut behind the genital pore are especially favorable for this purpose as they contain no part of the old reproductive system, unless the yolk glands are to be regarded as such.

Changes in the Old Part. — The changes in the old part of a regenerating planarian, which are evident in sections, are, — 1) Division of parenchyma cells, especially near the regenerating surface, and probable migration of the same, as such cells do not accumulate in the old part, nor do they seem to decrease in number. 2) Disappearance of food granules from the endoderm cells and desquamation of the vacuolar distal portion of many endoderm cells, the desquamated material appearing as rounded masses in the lumen of the digestive tract, usually after all food granules have disappeared. 3) Disintegration of yolk cells and consumption of the resulting fragments by endoderm and leucocytes. Portions of these yolk cells, some of them containing nuclei may be seen in the active endoderm cells, loose among the parenchyma cells, and in the grasp of the reserve endoderm cells and of leucocytes.

This phenomenon may be best observed in tail pieces of well fed specimens, from ten to twenty days after cutting. In a fifteen days specimen the yolk glands at the end of the tail are intact, while those farther forward are more or less broken down, and near the new part only rounded fragments are found.

This consumption of food material, much of it doubtless going to the regenerating part of the animal, and the decrease in bulk of the endoderm, probably account for the decrease in size of the original piece, and in part for the change in form of such specimens as are represented in Figs. *A* and *B*.

Conclusions.

1) The ectoderm of the regenerated regions of Planaria lugubris is formed primarily from the old ectoderm cells on the margin of the cut surface. Later increase in the new ectoderm is probably due to migration.

2) The new embryonic material which appears at a cut-surface results from multiplication of the so-called embryonic or parenchyma cells at that surface, and also probably from migration of similar cells from the old part where karyokinesis is abundant in such cells.

3) The muscle cells, gland cells and nerve cells of the new part are differentiated from the embryonic cells.

4) The new pharynx always appears in the new tissue on the border of the old part. It is differentiated entirely from the new embryonic tissue. A new pharynx is not formed when the old pharynx is left uninjured in the piece. If a part of the pharynx is cut off, it is completed by regeneration at the cut end.

5) Branches of the old digestive tract penetrate the new part for a short distance especially in the region of a new pharynx. Later growth of the digestive tract in the new part appears to be effected by differentiation of embryonic cells and addition of these to the branches which originate in the old part.

6) Excretory tubes from the old part penetrate the new part and there send out branches. Growth is probably by addition, as in the digestive tract.

7) Regeneration of the nervous system always occurs in direct connection with the nerve tracts of the old part. Fibres penetrate the new part and new nerve cells are differentiated from the embryonic cells.

8) The eyes appear to be a gradual differentiation of embryonic cells. The first to be observed are those which are most ventrally placed and others are added dorsally.

9) Auricular sense organs, probably organs taste or smell, are differentiated from the ectoderm rather later than the other organs.

10) The reproductive organs are the latest to regenerate. Tail pieces five weeks after cutting, show no signs of such organs.

11) Morphallaxis in this form appears to be a gradual lateral contraction and longitudinal elongation of the whole body, rather than any extensive migration of material. Diminished size of the old part in unfed specimens is amply accounted for by disappearance of food granules in the endoderm and of yolk cells from the yolk glands.

Woods Holl, Mass., Aug. 1901.

Zusammenfassung.

1) Das Ektoderm in den regenerirten Bezirken wird bei Planaria lugubris primär von den alten Ektodermzellen am Rande der Schnittfläche gebildet. Spätere Wachsthumsvorgänge im neuen Ektoderm beruhen wahrscheinlich auf Einwanderung.

2) Das neue embryonale (undifferenzirte) Material, welches an einer Schnittfläche erscheint, stammt von der Vermehrung der sogenannten embryonalen Binnen- oder Parenchymzellen an der Schnittfläche und auch von Einwanderung ähnlicher Zellen aus dem alten Gewebstheil, innerhalb dessen in den entsprechenden Zellen Karyokinesen sehr reichlich vorkommen.

3) Die Muskel-, Drüsen- und Nervenzellen des neuen Theiles differenziren sich aus den Parenchymzellen.

4) Der neue Pharynx erscheint stets in dem neuen Gewebe am Rande des alten Theils. Er differenzirt sich in seiner Gesammtheit aus den neuen Parenchymzellen. Ein neuer Pharynx wird nicht gebildet, wenn der alte in dem betreffenden Stück unverletzt blieb. Wurde ein Theil des Pharynx abgeschnitten, so wird er durch Regeneration am Schnittende wieder ergänzt.

5) Nachträgliches Wachsthum des Verdauungstractus in dem neuen Theile scheint durch Differenzirung von Parenchymzellen und deren Anfügung an die Zweige des Verdauungstractus, welche im alten Theile ihren Ursprung haben, zu geschehen.

6) Exkretionsröhren aus dem alten Theil dringen in den neuen Theil ein und verzweigen sich daselbst. Das Wachsthum beruht wahrscheinlich auf Zellanfügung wie beim Verdauungstractus.

7) Die Regeneration des Nervensystems kommt immer nur in direktem Zusammenhang mit den Nervensträngen des alten Theils zu Stande. Nervenfasern durchdringen den neuen Theil und Nervenzellen differenziren sich aus den Parenchymzellen.

8) Die Augen scheinen eine gradweise verschiedene Differenzirung der Parenchymzellen zu sein. Zuerst kann man die am meisten ventralwärts gelegenen bemerken, dann schließen sich ihnen andere dorsalwärts an.

9) Die aurikulären Sinnesorgane, möglicherweise Tast- oder Geruchsorgane, differenziren sich aus dem Ektoderm, und zwar erheblich später als die anderen Organe.

10) Die Fortpflanzungsorgane kommen bei der Regeneration zuletzt Schwanzstücke zeigen 5 Wochen nach dem Schnitt noch keine Spur von diesen Organen.

11) Die Morphallaxis bei dieser Species scheint mehr in einer schrittweisen lateralen Kontraktion und einer Verlängerung in der Längsachse des ganzen Körpers zu bestehen, als von irgend ausgedehnten Materialverschiebungen herzurühren. Zur Erklärung der Größenabnahme des alten Theils bei hungernden Exemplaren genügt reichlich das Verschwinden der Futterkörnchen im Endoderm und der Dotterzellen aus den Dotterdrüsen.

Literature.

MORGAN, T. H., '00, Regeneration in Planarians. Archiv f. Entwickelungsmech. X, 1. 1900.

BARDEEN, C. R., '01, On the Physiology of the Planaria maculata, with especial reference to the phenomena of Regeneration. Am. Journ. of Physiol. Febr. 1901.

FLEXNER, S., '98, The Regeneration of the Nervous System of the Planaria torvo (maculata) and the anatomy of the nervous system of the double-headed forms. Journ. of Morph. XIV. 1898.

Description of Figures.

Plate XVII.

Fig. 1 *a*. Portion of a longitudinal dorso-ventral section of a 16-hour piece showing method of closing in of ectoderm. *e* mass of embryonic cells; *d* dividing cell in new part; *f* ectoderm of old part, dorsal surface; *m* layer of circular muscles and pigmented region of old part; *r* rhabdite secreting cell; *i* dividing cell in old part; *n* old nerve tract.

Fig. 1 *b*. Ectoderm from another section of same piece showing groups of nuclei (*k*) and intercellular spaces.

Fig. 1 *c*. Ectoderm cells from same piece, closure not complete. ZEISS E, oc. 4, Cam.

Fig. 2. Embryonic cells in resting and division stages. Z. 2.00, oc. 4, Cam.

Fig. 3. Newly differentiated muscle cells. Z. 2.00, oc. 4, Cam.

Fig. 4. Mucous cells from new part. Z. 2.00, oc. 4, Cam.

Fig. 5. New endoderm cells, *a* two such cells found among embryonic cells in the new part near a branch of the digestive tract but entirely separate from it; *b* a cell taken from the end of the digestive branch from comparison. Z. 2.00, oc. 4, Cam.

Fig. 6. Horizontal section of a prepharynx piece, 5 d. 17 h. old, showing new pharynx and advance of old digestive tract into new part on either side of the pharynx and between th eeyes. BAUSCH and LOMB, ob. 1 in., oc. A, magnification 46 d. Cam.

Fig. 7. Longitudinal, dorso-ventral section from a 5 day tail piece. *e* small group of eye cells, two only in this section; *b* developing brain; *d* dividing cells in the new part; *i* karyokinesis in old part; *p* new pharynx;

c **pharynx chamber;** *m* **prospective mouth;** *a* **digestive tract. B. and L., ob. 1 in., oc. C. mag. 80 d. Cam.**

Fig. 8. **Two longitudinal dorso-ventral sections** from a piece cut through the **anterior part of the** pharynx **and fixed after** 46 hours. *o.p* old pharynx: *n.p* **new** pharynx.

Fig. 9. New pharynx forming in front of a portion of the genital chamber. *p* **new** pharynx; *c* part of old genital chamber. B. and L., ob. 1/2 in., oc. C, **mag. 165** diam. Cam.

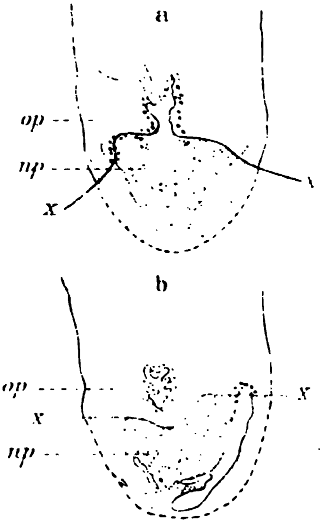
Fig. 10. **Sections of** excretory **tubes** in the new **part.** *a* and *b* **cross** sections; *c* **longitudinal** section showing branching end **in** the new **part.** ZEISS 2.00. **oc. 4, Cam.**

Fig. 11. **Unipolar** and **bipolar nerve** cells. Z. 2.00, oc. 4. Cam.

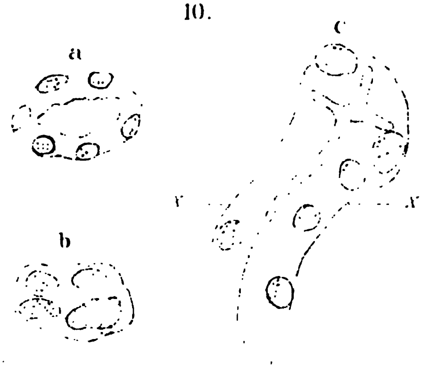
Fig. 12. **Section** of **regenerating** eye 6 days. Z. E. oc. 4, Cam.

Fig. 13. Auricular sense organ from new head region of a 10 day tail piece. Z. 2.00. oc. 4. Cam.

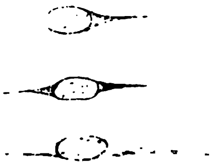
8.



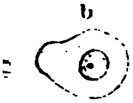
10.



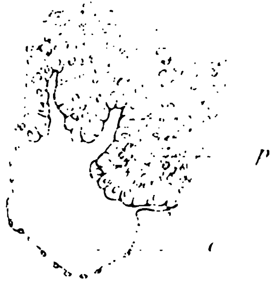
11.

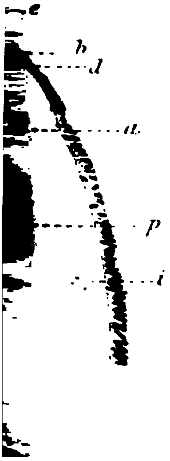


5.

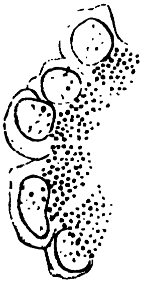


9.

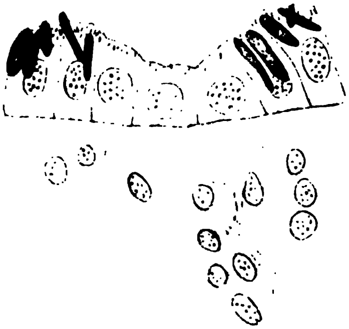




12.



13.



The Proportionate Development of Partial Embryos.

By

T. H. Morgan.

With 1 figure in text.

Eingegangen am 16. August 1901.

or embryonic digestive tract — since it is the first[1]) organ that develops out of the blastula, and by counting the number of cells in the archenteron of the whole and of the »partial« embryos I attempted to find out if the smaller embryo makes use of the same number of cells as does the whole embryo, or of only half as many, etc. If the first method is adopted, then as the cells in the archenteron are as many and as large as in the full-sized embryo the digestive tract must also be as large, and it will be relatively twice too large for the rest of the body, and hence the embryo will not be a miniature copy of the whole embryo. If the other alternative is followed, then one half the number of cells characteristic of the entire embryo will produce an archenteron of the same relative size, although the cells will be relatively twice too large. The results of a large number of counts that I made of the cells in the archenteron of the small embryos seemed to show that the number turned in was generally greater in the $^1/_2$ embryo than half the entire number and greater in the $^1/_4$ embryo than one-fourth the whole number. I concluded that the small embryo tended to use the same number of cells to produce its organs that is characteristic of the entire embryo. There are several considerations that make this conclusion of doubtful value; for instance, a), the delay in the development may make it possible for the cells in that part of the wall from which the archenteron will form to increase more rapidly in number as they turn in, than do the cells in the normal embryo, and if so, while their number would be larger for a given stage, the cells themselves would be smaller; b), there is no exact moment at which the gastrulation can be said to be completed unless we choose some such arbitrary limit as the formation of the gut-pouches, or the contact of the inner end of the archenteron with the stomodaeum, hence there is a difficulty in comparing identical stages, although this can be done approximately; c), lastly great variability was found to exist in the actual number of cells turned in to form the archenteron, although the highest numbers agreed with that characteristic of the entire embryo.

My conclusion that the number of cells in the archenteron in the smaller embryos was proportionately larger, and approximated to that in the entire embryo seemed to be supported also by the observation that very often the number of cells in archentera that were not even completely finished was nearly the normal number and much greater than half the number; and moreover in many cases the archenteron was much larger in proportion to the rest of the body in the small embryo than in the normal one.

---

[1]) Excepting the mesenchyme which however only forms a skeleton at a later stage.

Previously I had examined the number of cells in the $^1/_2$ embryo of a teleost — Fundulus —, and had found also evidence that the number of cells characteristic of the adult went to form the eye, nerve cord, etc., of the $^1/_2$ embryo. Later I examined the number of cells in embryos of Amphioxus that had developed from isolated $^1/_2$ and $^1/_4$ blastomeres. In these the total number of cells in the $^1/_2$ embryo was found to be half of the number¹) in the normal embryo, and in the $^1/_4$ embryo one fourth the total number. Cross-sections of these embryos seemed to show that more than two-thirds the normal number of cells were present in cross-sections in some of the organs of the $^1/_2$ embryo, and more than one half to make the organs of the $^1/_4$ embryo.

The results of my study of the number of cells invaginated by whole and partial embryos of Sphaerechinus showed as stated above that a proportionately larger number are turned in by the smaller embryos. The whole embryo of Sphaerechinus contains somewhat over 500 cells in the wall of the blastula, and, of these, 50 cells are invaginated to make the archenteron.

In a very small embryo, from an egg-fragment, containing only 57 nuclei in the outer wall, there were 22 nuclei in the archenteron, in another, with 56 nuclei in the outer wall, there were 36 nuclei in the archenteron. If these embryos had turned in only one-tenth the entire number present in the blastula they would have used only eight to ten cells, but the results show, that about twice as many in one case, and three times as many in the other were invaginated.

Another embryo of Sphaerechinus derived from a $^1/_8$ blastomere had 79 cells in the wall and turned in 20 to 25 in the archenteron which at the time was only about two-thirds completed. If the proportionate number had been invaginated only ten cells would have been used to form the entire archenteron. Another embryo that developed from a fragment of the blastula wall contained only 31

---

¹) Through an unfortunate mistake or error the number of cells given (on page 379 of my paper) for the $^1/_2$ larva was too great, although the data given show the number of cells to have been one half the total number. DRIESCH has also called attention to this error.

10) Die Fortpflanzungsorgane kommen bei der Regeneration zuletzt. Schwanzstücke zeigen 6 Wochen nach dem Schnitt noch keine Spur von diesen Organen.

11) Die Morphallaxis bei dieser Species scheint mehr in einer schrittweisen lateralen Kontraktion und einer Verlängerung in der Längsachse des ganzen Körpers zu bestehen, als von irgend ausgedehnten Materialverschiebungen herzurühren. Zur Erklärung der Größenabnahme des alten Theils bei hungernden Exemplaren genügt reichlich das Verschwinden der Futterkörnchen im Endoderm und der Dotterzellen aus den Dotterdrüsen.

Literature.

Morgan, T. H., '00. Regeneration in Planarians. Archiv f. Entwickelungsmech. X, 1. 1900.

Bardeen, C. R., '01. On the Physiology of the Planaria maculata, with especial reference to the phenomena of Regeneration. Am. Journ. of Physiol. Febr. 1901.

Flexner, S., '98. The Regeneration of the Nervous System of the Planaria torva (maculata) and the anatomy of the nervous system of the double-headed forms. Journ. of Morph. XIV. 1898.

Description of Figures.

Plate XVII.

Fig. 1 *a*. Portion of a longitudinal dorso-ventral section of a 16-hour piece showing method of closing in of ectoderm. *e* mass of embryonic cells; *d* dividing cell in new part; *ec* ectoderm of old part, dorsal surface; *m* layer of circular muscles and pigmented region of old part; *r* rhabdite secreting cell; *i* dividing cell in old part; *n* old nerve tract.

Fig. 1 *b*. Ectoderm from another section of same piece showing groups of nuclei (*k*) and intercellular spaces.

Fig. 1 *c*. Ectoderm cells from same piece, closure not complete. Zeiss E, oc. 4, Cam.

Fig. 2. Embryonic cells in resting and division stages. Z. 200, oc. 4, Cam.

Fig. 3. Newly differentiated muscle cells. Z. 200, oc. 4, Cam.

Fig. 4. Mucous cells from new part. Z. 200, oc. 4, Cam.

Fig. 5. New endoderm cells, *a* two [illegible] found [illegible] in the new part near a branch [illegible] digestive tract [illegible] from it; *b* a cell taken fr[illegible] the [illegible] parison. Z. 200, oc. 4.

Fig. 6. Horizontal section [illegible] pharynx and adva[illegible] of the pharynx [illegible] magnification 46 [illegible]

Fig. 7. Longitudinal [illegible] group of eye [illegible] ing cells in [illegible]

c pharynx chamber; m pharyngeal [illegible]
ob. 1 in. oc. C. mag. 80 d. Cam. [illegible]

Fig. 8. Two longitudinal dorso-ventral [illegible] anterior part of the pharynx and [illegible] n.p new pharynx.

Fig. 9. New pharynx forming in front of [illegible] p new pharynx; c part of old genital chamber [illegible] oc. C. mag. 150 diam. Cam.

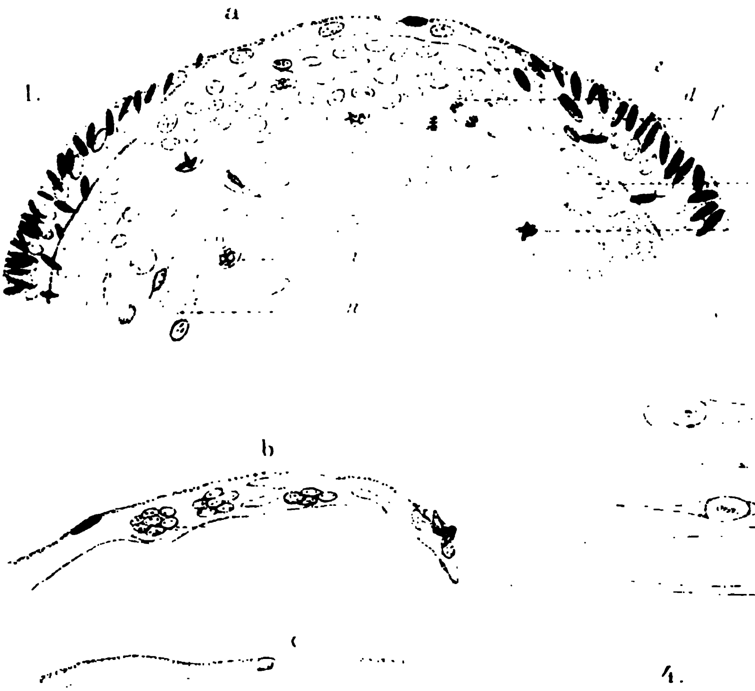
Fig. 10. Sections of excretory tubes in the new part [illegible] a longitudinal section showing branching and [illegible] in the new part [illegible] oc. 4, Cam.

Fig. 11. Unipolar and bipolar nerve cells. Z. 2.00, oc. 4, Cam.

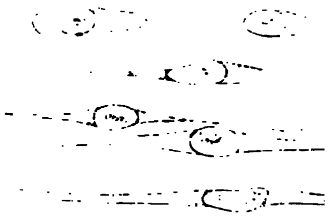
Fig. 12. Section of regenerating eye 8 days. Z. F, oc. 4. Cam.

Fig. 13. Auricle [illegible] from new head region of a 10 day old [illegible] Z. 2.00, oc. 4. Cam.

1.

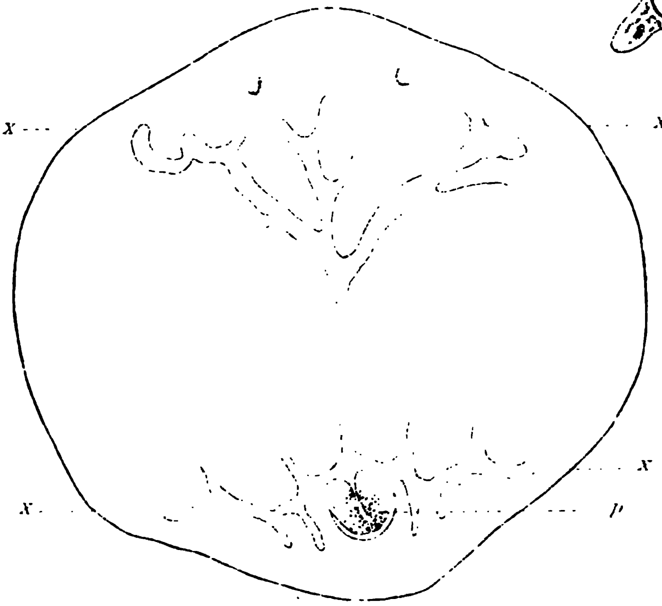


3.



4.

6.



7.

Wilhel

differently in regard to the total number of cells in the same embryo. I doubt, however, if this solution would be entirely satisfactory.

There is a further suggestion that may account in part for the larger number of invaginated cells in the small larva, viz., the delay that takes place in the gastrulation of the smaller pieces. There are some facts that seem to support this conclusion. The larger partial embryos gastrulate sooner than do the smaller ones, and in many cases the disproportion in the number of cells turned in is less than in the smaller embryos, i. e., although they may more often have the whole number, yet this represents a smaller proportionate increase than that found in the small embryos. Further it is by no means improbable that after a long delay the cells in the endodermal plate of the small larvae may begin to divide and then gastrulate. The cells turned in correspond, therefore, to a relatively smaller number of cells in the blastula wall. This process would be different from that which takes place in the normal embryo, and while accounting for the larger number of cells in the small embryo, it also shows, at the same time, that they may come from a proportionate number of cells of the blastula.

That this hypothesis does not give a completely satisfactory solution of the difference is found in an examination of the size of the archenteron in the very small embryos. In the figures that I have given it will be easily seen that the archenteron is often very much too large for the size of the piece. There can be no doubt that many of the very small embryos present this characteristic. This condition would not exist if the gastrulation took place by the later division of a proportionate number of cells in the endodermal plate of the smaller embryo.

Closely connected with the questions relating to the number of cells in embryos from isolated blastomeres are those relating to the method of development of embryos from fragments of the unsegmented egg. The two problems are by no means necessarily identical, or at least we could not have assumed them to be so on a priori grounds, since we had no evidence to decide whether the whole nucleus of an egg fragment would run through the same number of division characteristic of the undivided egg, or whether the number of division would be in proportion to, and limited by the amount of protoplasm contained in the fragment.

The results of my examination of the development of fragments

The Proportionate Development of Partial Embryos.

By

T. H. Morgan.

With 1 figure in text.

Eingegangen am 16. August 1901.

were cut into sections at different, but corresponding stages, in the hope of finding out whether the smaller embryo contains as many cells as the larger embryo, etc. It has been so difficult to decide with certainty in regard to this question, that I prefer not to commit myself until I can procure new material in which a greater difference in the sizes of the eggs may make it easier to solve the problem. When it is remembered that a cell having half the volume of another, has a diameter in the ratio of 4 to 5 it will be seen how difficult the problem becomes in an embryo having cells of all sorts of irregular shapes.

It may, perhaps, only lead to further difficulties to bring into this connection the results of other experiments that seem to offer a parallel case. It has been shown that small pieces of the stem of Tubularia, that are shorter than the region from which in larger pieces the hydranth is made, produce smaller hydranths at one end of the piece although not proportionately smaller hydranths. In other words although the hydranth is reduced, it is by no means reduced in proportion to the size of the very small pieces. DRIESCH has found that if short pieces are taken from the region just behind the old hydranth, the entire piece is very often transformed into a hydranth only, or even into only a part of a hydranth. There is a »tendency« in this part of the stem to produce not a proportionate structure, but a larger one in relation to the size of the piece, and I think, we may add, there is a tendency to form the whole-sized structure or the whole-sized distal part of the hydranth. Since these structures are full-sized they will use proportionately too great a number of cells. This case is, it seems to me, parallel to that of the small embryos.

A somewhat similar result has been found by PEEBLES in the regeneration of very tiny pieces of Hydra. These pieces do not form the total number of tentacles characteristic of the full-sized hydra, but only one, two, three, or four tentacles. If the tentacle ring had been organized on a proportionately smaller scale we should expect six to eight tentacles to appear of very small size, but as this never happens it looks as though tentacles of only a certain size can be formed and hence there is room for fewer of them, i. e., each uses up too much material, or too many cells of the old part.

These examples are, it is true, from another field, but they show us nevertheless that the problem of the formation of smaller organisms may involve other, quantitative factors, than simply the production of proportionate organs of smaller size. I have pointed out that these results seem to indicate that the cells as units play only a secondary part in the moulding of the organs of the metazoon body. The development of the typical form by small embryos having only a half or a fourth the total number of cells points to this conclusion, and the facts discovered by CONKLIN, viz., that giants and dwarfs are produced not by larger and by smaller cells, but by more or fewer cells, leads to the same conclusion. I need scarcely add that we are not, for this reason, justified in ignoring entirely the cells in studying these processes, for the proportionately enormous size of the cells in the very small larvae may still be an important factor in the development, or lack of development of the embryo. But the main fact seems clear, that the organisation of the metazoon, so far as the formative processes are concerned involves the protoplasm as a whole, and is not the outcome of the action of cells as units, although the individual cells as centers may act as boundaries to the action of the formative forces, i. e., in the production of organs a cell is either entirely involved or not at all.

II.

The preceding summary was written a year ago, and in order to clear up some of the points that have been discussed it was necessary to obtain new material. During June of the present year I went to Beaufort, N. C., to examine the problem of the number of cells invagination in the embryo of the sea-urchin, Toxopneustes variegatus. Thanks to the ample opportunities offered by the U. S. Fish Commission I obtained the necessary material. I gladly express my obligations to Prof. H. V. WILSON in charge of the Station for many courtesies extended to me during my stay.

The blastomeres were separated by HERBST'S method. The eggs were fertilized, and within two to five minutes afterwards were shaken in a tube until they were freed from the membrane. The eggs, after settling, were thrown into HERBST'S calcium-free sea water. They remained in this solution until they had reached the two, four or eight-cell stage. They, were then removed, gently shaken in order to separate the blastomeres and put into normal sea water. In this way, as DRIESCH has shown, large numbers of isolated blastomeres can be obtained. The method, I can also attest, is immensely superior to the former one of violently shaking the membraneless eggs to separate the blastomeres.

It was evident that in order to settle the difference between my former results and those of Driesch it would be necessary to preserve continuous series of embryos of the same lot. I should add that I have preserved only the partial embryos that rose to the top of the water, as do the normal embryos. These, as experience has taught me, are much more apt to develop into later stages than are the abnormal embryos that swim around on the bottom. The embryos were killed in a corrosive-acetic solution, stained in Orth's picro-lithium carmine and mounted in balsam.

In the first series, here chosen for description, the gastrulation was completed in the whole embryos at 11 a. m., and even in some of the $^1/_2$ and the $^1/_4$ embryos, although in only a few of these. The whole embryos had about 955 cells (as indicated by the number of nuclei) in the wall and about 150 in the archenteron. The $^1/_2$ embryos contained in the archenteron, in five cases, 96, 85, 66 (not finished), 56 (not finished). The $^1/_4$ embryos contained in the archenteron 49, 57, 40, 40 (incomplete). These results show that in the archenteron of the partial embryos these are at this time about a half and a fourth respectively of the number of cells in the archenteron of the whole embryo.

Two hours and a half later the whole embryos have about 180 to 190 cells in the archenteron. The $^1/_2$ embryos, in five counts, 96, 85, 90, 95, 77 +, and the $^1/_4$ embryos 60, 75, 56, 63, 60, 65 cells in the archenteron.

Three hours later there were, in two counts, 268 and 283 cells in the archenteron of the whole embryo. The $^1/_2$ embryos contained in three counts 148, 106, 92, 105 cells and the $^1/_4$ embryos contained 70, 73, 72, 70, 60, 36 ($^2/_3$ gastrulated), 60 (197 cells in wall), 27 (completely gastrulated with 200 cells in wall), 46 ($^1/_2$ gastrulated, 192 cells in wall).

These results show that there is a steady increase in the number of cells in the archenteron both in the whole and in the partial embryos. If the number of cells in the archenteron of the whole and of the partial embryos of the same age in point of time is compared it is found that the $^1/_2$ and $^1/_4$ embryos contain about a half and a fourth of the number present in the whole embryo. On the other hand it appears that a relatively larger number of cells is invaginated in some of the partial embryos than in the whole embryos, and, moreover, in comparison to the number of cells in the outer wall, the partial embryos, especially in those that gastrulate late, turn in proportionately more cells than are at first turned in by the whole embryos.

There is a further point that should be remembered. A few of the partial embryos gastrulate almost as soon as do the whole embryos and there can not be any doubt that in these the number of cells continues to increase from hour to hour. Embryos of this kind will also appear in the later lots, but nevertheless most of the partial embryos in the later lots represent individuals that have gastrulated later, and since practically all of the partial embryos of the later series have in the archenteron more than a half or a fourth or an eighth of the whole number of cells that is first invaginated in the whole embryo there can be no doubt that these delayed partial embryos turn it at first proportionately a large number of cells. The explanation of this is found in the continuous process of cell division by means of which a somewhat larger number of endodermal cells are present when invagination begins.

A second series of embryos, selected for description, includes earlier stages than the last series. The whole blastula that is about to gastrulate has about 800 cells in the wall (including those that are to be invaginated). The $^1/_2$ blastula contains at this time (8 30 a. m.) from 350 – 370 cells and the $^1/_4$ blastula from 180—218 cells. These figures show that the partial embryos have respectively a half and a fourth the whole number of cells.

Two hours later gastrulation had taken place in the whole embryos. They were found to have in one case about 1000 cells in the outer wall, and in three cases 117, 120, 130 nuclei in the archenteron. Some of the $^1/_2$ embryos that had gastrulated contained in five counts 76, 68, 76, 67, 65 ($^2/_3$ gastrulated) nuclei in the archenteron.

An hour later the number of cells in the wall of the whole gastrula was in one case 1350 with 250 cells in the archenteron, and in another case 1440 with 265 in the archenteron. Others had in the archenteron 127, 180, 170, 145, 176 cells. The $^1/_2$ gastrulae had 120 (with 463 in the wall), 96, 97, 92, 97, 86 nuclei in the archenteron.

After another hour the number of cells in the wall of one whole gastrula was 1510 cells (with 313 in the archenteron). Other counts gave 290, 353 in the archenteron. The $^1/_2$ gastrulae contained at this time 112, 134, 140, 130, 196 nuclei in the archenteron. The $^{1}/_{4}$ gastrulae had just begun to gastrulate and in some of these the archenteron did not seem to have been completed. Four counts gave 33 nuclei in the archenteron (and 240 in the wall). 36 (with 193 in the wall), 50 (with 250 in the wall), 44 (with 214 in the wall).

The results of this second series bear out the conclusions from the first series. Other series were not as good as these in all respects, but so far as they go they give similar results.

Figs. *A*—*C'*.

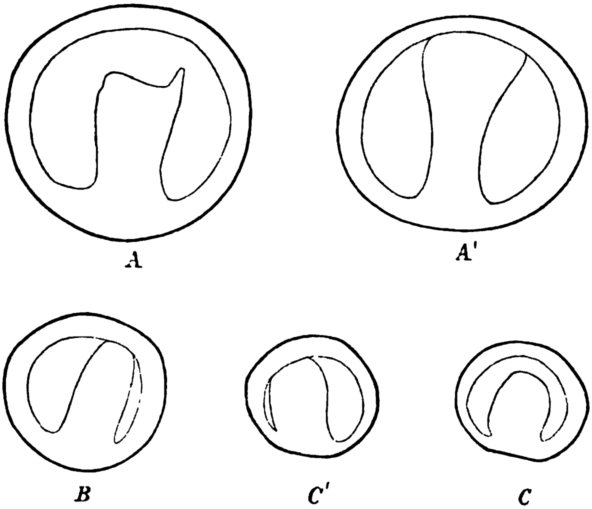


Fig. *A*. Whole embryo about two-thirds gastrulated. Fig. *A'*. Whole embryo, gastrulation completed. Fig. *B*. Half-embryo, gastrulation completed. Fig. *C*. One-fourth embryo. Fig. *C'*. One-fourth embryo, showing excentric archenteron.

A fact that I had formerly observed is very apparent in these new preparations, viz., that the archenteron in most of the partial embryos is proportionately too large for the size of the embryo[1] and this is especially obvious, as shown in Figs. *A*, *A'*, *B*, *C*, *C'*, in the $^{1}/_{2}$ and $^{1}/_{4}$ embryos. Some of Driesch's figures seem also to me to show the same thing. The result appears to be due in part to the proportionately large number of cells in the archenteron of the smallest partial embryos. The archenteric cells appear to divide oftener than the cells in the wall, and if the cells enlarge after each division the larger size of the archenteron in the retarded embryos can be accounted for. The proportionately smaller enlargement of the wall of the partial embryos may also make the difference more apparent.

---

[1] One apparent exception to this is given in the first series. In this case the partial embryo appeared to have a proportionately formed digestive tract that contained 27 cells and the wall 200 cells. The numbers bear the ratio characteristic of the whole embryo.

Another fact that is obvious in all these embryos is that the gastrulation of the $^1/_2$ and $^1/_4$ embryos is quite excentric as shown in Figs. *B*, *C'*. This excentricity may be easily overlooked since the side towards which the archenteron turns, being heavier, is generally turned away from the observer looking at the embryos from above, Fig. *C*, but if the embryos are rotated the excentric position of the archenteron is readily detected, Fig. *C'*. Thus the partial embryos are not at first simply whole embryos of small size, but show traces of their origin as a part of the egg. It has been observed by all those who have examined the cleavage of the isolated blastomeres of the sea-urchin that the cleavage is, as a rule, incomplete 1). It has been supposed that this incomplete development extended only to the cleavage, but it seems to me very probable that the excentric position of the archenteron in the partial embryos of Toxopneustes is also the outcome of the unsymmetrical structure of the blastomeres. The rapid development of the embryo in this form — gastrulation beginning in warm weather within fifteen hours after fertilization — may in part account for the result. The excentric position of the archenteron in this case seems to be similar to that found in the formation of the »stomach« in the ctenophore egg as described by DRIESCH and MORGAN.

BOVERI's recent study 2) of the egg of Strongylocentrotus lividus has shown that the micromere pole lies opposite to the position taken by the segmentation nucleus — a fact that WHITMAN and I had also observed in 1894. BOVERI has also shown that the whole micromere hemisphere is invaginated to form the archenteron. DRIESCH's results had already made it highly probable that the archenteric pole is the micromere pole, and BOVERI's results make this certain. BOVERI states that although his results show that there is a definite relation between the parts of the egg and the gastrula, yet his own observations (as do also those of DRIESCH and myself) show that while this may be true yet pieces of the egg may still

---

1) Except in one case in Toxopneustes described by E. B. WILSON in which total cleavage was observed.

2) Verhandl. d. phys.-med. Gesellsch. zu Würzburg. N. F. XXXIV. 1901.

gastrulate that have come from the hemisphere that is opposite the micromere pole. We see then that even in cases with a perfectly definite type of cleavage that give rise to embryos having definite relations to this cleavage there is no absolutely necessary relation between the two, for if the conditions are changed the relation may also be altered or at least parts of the egg may invaginate that do not do so under ordinary circumstances. Nevertheless the pre-existing protoplasmic relations in the segmenting egg appear to have an important influence on the formation of the normal embryo, and such a relation accounts for, I think, the excentric position of the archenteron in the $^1/_2$ and $^1/_4$ embryos of Toxopneustes.

Summary.

1) Those $^1/_2$ and $^1/_4$ embryos of Toxopneustes variegatus that gastrulate at nearly same time as do the whole embryos invaginate about one half and one fourth the number of cells invaginated by the whole embryos.

2) Those $^1/_2$ and $^1/_4$ and $^1/_8$ embryos, that gastrulate later than the whole embryos, invaginate proportionately a larger number of cells. The explanation of this is that the number of cells slowly increases in the partial blastulae so that those that gastrulate late turn in more cells than those that gastrulate early.

3) The size of the archenteron in the late partial embryos, especially in the $^1/_4$ and $^1/_8$ embryos, is generally proportionately larger than in the whole embryos.

4) The archenteron in the $^1/_2$ and $^1/_4$ embryos is excentric in position which seems to be due to the unsymmetrical organisation of the isolated blastomere.

5) The number of cells invaginated in late gastrulae and, in general, in the late smallest gastrulae, as compared with the number in the outer wall is greater in the partial embryos than in whole embryos.

Zusammenfassung.

1) Diejenigen Halb- und Viertelembryonen von Toxopneustes variegatus. welche die Gastrula annähernd zur selben Zeit bilden wie die ganzen Embryonen. stülpen etwa die Hälfte oder ein Viertel der Zellenanzahl ein wie die ganzen Embryonen.

2) Diejenigen Halb-, Viertel- und auch Achtelembryonen, welche die Gastrula später als die ganzen Embryonen bilden, stülpen eine verhältnismäßig größere Zahl von Zellen ein. Es erklärt sich das aus dem allmählichen Wachsthum der Zellenanzahl in den Theilblastulae, so dass die spät die Gastrula bildenden mehr Zellen einstülpen als die frühzeitig gastrulirenden.

3) Die Größe des Urdarmes in den Theilembryonen, speciell in den Viertel- und Achtelembryonen, ist in der Regel verhältnismäßig bedeutender als in den ganzen Embryonen.

4) Der Urdarm in den Halb- und Viertelembryonen besitzt eine excentrische Lage, welche von der unsymmetrischen Organisation der isolirten Blastomeren herzurühren scheint.

5) Die Anzahl der eingestülpten Zellen bei den spät gebildeten Gastrulae und im Allgemeinen bei den kleinsten Gastrulae ist im Vergleich mit der Zellenzahl der Außenwand in den Theilembryonen größer als in den Ganzembryonen.

Reprinted from the American Journal of Physiology.
VOL. VI. — MARCH 1, 1902. — NO. VII.

THE INFLUENCE OF TEMPERATURE, ODORS, LIGHT AND CONTACT ON THE MOVEMENTS OF THE EARTHWORM.

BY AMELIA C. SMITH.

[*From the Laboratory of Biology at Bryn Mawr College.*]

INFLUENCE OF TEMPERATURE.

ALLOLOBOPHORA FŒTIDA, the common manure worm, lives in damp soft soil, and in heaps of well-rotted or decomposing manure. In the laboratory the animals were kept in tin pails half full of manure, where they lived all winter in apparently perfect health and produced great numbers of cocoons and eggs. The average temperature of this manure was 18°C., a little below the room temperature.

When a number of worms were placed on a wet pane of glass, and the latter slowly warmed, the worms did not creep away from the warm place until it became uncomfortably hot to the finger, although when the heat was intense enough to cause the water on the plate to steam, the worms crawled rapidly away, and many died after a brief exposure to such temperatures.

Preliminary experiments to determine the effect of temperature were made with the following apparatus. A large sheet of glass twelve inches square was supported at two corners at a height of eleven inches above the table. It was kept sprinkled with water, and two thermometers were laid on it. An alcohol lamp stood on the table beneath the plate, and by raising or lowering this lamp, and by moving it under different portions of the glass, the temperature of the plate was varied as much as was necessary. The worms were placed in the centre of the plate either over the flame or at a little distance from the hottest area. If a number of worms are put down near each other they soon crawl together and coil into a heap. Such a heap of worms on the plate is not disturbed by a rise of temperature from 20° to 30° C. If the temperature of the plate rises above 30° C., where the worms lie, there is at first more active squirming, which changes to a streaming if the temperature reaches 33° to 34° C.

Individuals rarely separate from the heap, but the whole mass moves away from the heated area. If the flame is brought nearer while this streaming is in progress, the rate is greatly accelerated, until 36° to 37° C. is reached, while temperatures from 36° to 40° C. prove fatal.

These experiments are open to the objections that the worms are crawling over an unwonted surface, exposed to the air, and the apparatus was accordingly modified as follows. A half-inch layer of manure was spread between two plates of glass twelve inches square. The manure was moistened until it was very sticky and was spread evenly, but not pressed down. Three thermometers (t^1, t^2, t^3) were thrust into the manure, one at each of the free corners, and one in the middle. The apparatus was supported by two corners as before; an alcohol lamp under one free corner served to raise the temperature, and a dish of ice-water, with ice floating against the lower plate, to lower the temperature. Readings from the three thermometers were taken from time to time, and when there was a marked change of temperature the upper plate was removed and the manure was divided into three sections (each containing a thermometer) by transverse cuts with a ruler. The worms in each section were then counted.

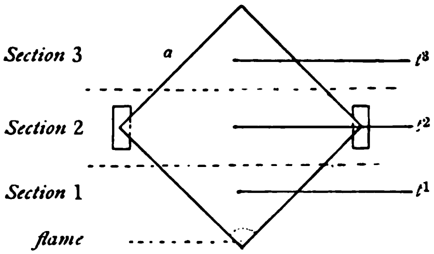


FIGURE 1.

Experiment 1. — The apparatus was that described above, heated at one corner (Section 1) for ninety minutes by an alcohol lamp placed just below the plate. Twenty worms were introduced into the manure at *a* (Fig. 1). The results are given in Table I. (The fact that t^1 registered a lower temperature at 12 M. than at 10.50 A. M. is due to the circumstance that the lamp burned out a few minutes before the cover was removed.)

Experiment 2. — Thirty-two worms were put into the manure in the centre of the plate. Below the middle of the lower plate was a dish of ice-water with a large lump of ice floating against the plate. The experiment continued until t^2 (Fig. 1) registered its lowest temperature and began to rise. The results are given in Table II.

TABLE I.

Section.	Temperature at 10.30. C.	Temperature at 10.50. C.	Temperature at 12.00. C.	Position of worms at 12.00
1	41°	51°	48°	0
2	25°	30°	34°	5
3	23°	28°	30°	15

TABLE II.

Section.	Temperature at beginning (10.25). C.	Temperature at end (11.40). C.	Position of worms at 11.40.
1	18.0°	19.0°	7 = 22%
2	17.0°	8.5°	12 = 37%
3	17.5°	19.0°	13 = 41%

Experiment 8. — Thirty-two worms were put into the manure over t^1 (Fig. 1). Under Section 1 was the flame, under Section 3 the lump of ice. The results are given in Table III.

TABLE III.

Section.	Temperature at beginning. C.	Position of worms at beginning.	Temperature at end. C.	Position of worms at end.
1	18°	32	70°	3 (dead)
2	20°	0	30°	21
3	20°	0	10°	8

If the heat is applied only to the tail, the worms crawl rapidly away in a straight line. When the head touches a hot place or object, the worm contracts sharply, turns aside or sometimes crawls backward for an inch or two. If the heat is too intense the worm squirms violently before crawling away. If the entire worm is laid on a warm place (30°–35°C.) the worm crawls as quickly as possible. That these differences in reaction are due less to differentiation between the two ends of the body than to the fact that the animals habitually move forward rather than backward, and consequently if stimulated on the head end are obliged to progress in a less usual way to escape the stimulus, is evidenced by the following facts. Worms from which three, four or five anterior segments have been removed respond to the stimulus of heat like normal worms. If beheaded and normal worms are placed together on a hot area, they cannot be distinguished by their reactions.

Summary. — The preceding experiments point to the conclusion that a rise in temperature of ten degrees (*i. e.*, 18° - 28°C.) above that in which A. fœtida lives does not cause the retreat of the worms. A rise from 29° to 35° causes retreat, and violent squirming if the animal is retained in this temperature, while a rise from 35° to 40° is fatal, although such a temperature rarely causes progressive crawling. It is interesting to note that when a number of worms are coiled together, they respond not as individuals, but as a whole, the movement resembling that of a single organism.

INFLUENCE OF ODORS, AND EFFECT OF DRYING.

In his book on "The Formation of Vegetable Mould by the Action of Earthworms" Darwin describes in the section on "Habits," some experiments in which he tested the sense of smell of earthworms. From their finding bits of food buried in the soil at a distance from their burrows, he concludes that earthworms possess the sense of smell in a slight degree. The behavior of Allolobophora fœtida when accidentally dropped near a pile of wet fresh sawdust led to some experiments with cedar oil, turpentine, ether, and similar substances. If a glass rod dipped in cedar-oil is held at a distance of one quarter of an inch from the head, there is usually a slight recoil, although the reaction is not constant. Xylol similarly held one quarter to one eighth of an inch from the head causes a slight recoil, and if the stimulation continues the worm turns and crawls away. Turpentine causes a similar recoil and change of direction. Either cedar-oil, xylol, or turpentine, if allowed to touch any part of the body, causes a violent squirming and the production of the offensive-smelling yellow secretion to which A. fœtida owes its name. At the same time the worm retreats as quickly as possible; if touched on or near the head it squirms backward for a short distance before turning aside, but if touched on the tail-end, it crawls forward at once, squirming actively at the same time. A drop of ether on a glass rod held one quarter of an inch from the head produces a marked reaction. The worm recoils instantly and crawls quickly backward, usually accompanying this retreat by lifting fifteen or twenty anterior segments into the air and waving them about. If the worm is pursued with the rod, violent squirming results, and by quickly coiling and uncoiling, in opposite directions, the animal springs clear from the paper several times in succession. If the rod is touched to any part of the worm the squirming and springing are at first very violent, but after several springs the worm stiffens. When these substances are held near the tail (provided the animal is not bent so that the head also is affected) there is either a faint response or none. In this respect there is a slight individual difference.

When these worms are removed, for a longer or shorter time, from the manure in which they live, and are then returned, they at once burrow into the manure. When placed on soil with no admixture of manure, they crawl over the top for a time before burrowing. If removed from the manure to wet filter paper or some similar damp surface, they can crawl over it for an hour without exhibiting any sign of discomfort[1] except restlessness, which is probably due to the unwonted light. If, however, they are placed on a dry surface, the skin becomes dry in a few minutes, the worms crawl more and more slowly, and frequently lift half the body into the air, waving it from side to side with a peculiar rotary motion. At the end of two or three minutes the irritation has become so great that the worm squirms violently, produces the offensive odor, and after several springs it stiffens. If returned to moisture at this time the worms recover. If they are allowed to remain dry, one or two additional springs are made, the animals becoming stiffer after every such effort, and death soon follows. The early stages of this drying up are easily recognized by the "tight" appearance of the skin and the apparently unwarranted irritability, for at this period the slightest touch produces violent squirming.

The sensitiveness to drying led to some experiments to determine

---

[1] In using words like "discomfort" and "choice," which occur throughout this paper, it is perhaps necessary to state expressly that no psychic element is thereby introduced. The words are used to avoid long and awkward circumlocutions, and with this understanding furnish a readier, simpler, and more direct vocabulary for describing the reactions of these animals than more exact but longer expressions.

whether the animals perceive moisture at a short distance from the body, and whether any factor other than contact is involved in their burrowing. A twelve-inch square of filter paper laid on a plate of glass was made very wet in the centre. In a few minutes there was a wet spot, surrounded by a damp ring, with an outer margin of dry paper. When worms were laid on the dry paper and allowed to move freely they crawled around and around the wet spot with its damp ring until the skin dried, but unless the head and a few segments crossed the edge, they never crawled into the wet places. They even crawled along the edge of the damp ring without turning into it. But if the worm once crossed the edge of the damp region it crawled in at once, and remained in the wettest part, even when exposed to full sunlight.

The filter paper was now completely moistened, and in the centre was a little heap of tiny shreds of wet filter paper. The object of this arrangement was to provide a wet heap into which the worms could crawl if contact and moisture alone stimulated them to burrow. When laid on the paper the worms crawled around the heap as before. If their course accidentally brought them against the heap, they crawled over it without burrowing. In one case a worm burrowed a short distance, then withdrew and crawled over the top. The heap of paper was then moistened with a decoction of the manure in which the worms lived, instead of with tap-water. The results showed an interesting variation. The worms were no more apt to find the heap than before, but if they did find it, they burrowed at once and remained there. Seven out of ten burrowed at once, and only one left the heap after entering. A heap of wet sawdust was now substituted for the paper, but the worms turned aside from this as soon as the head came in contact with it. They not only did not enter, but did not crawl over the heap, except in one case out of ten. Finally a small pile of manure was put in the centre of the plate, and the worms were allowed to crawl around it. As in the other cases, there was no indication that the manure attracted the worms from a distance, for unless gently headed toward it by the experimenter they crawled by it even when very close. One worm turned aside just before reaching the manure, and skirted the edge in such a way that in the middle of the body the back touched the manure, and still the worm did not enter the heap. But if the head touched the manure, or any outlying fragment thereof, the worms burrowed at once, and remained in it until removed. When the tail was in contact with manure I saw no tendency to burrow, although when worms in manure are disturbed, they travel through it forward or backward indifferently.

Summary. — The above experiments indicate that while these animals are not stimulated by certain odors at a distance from the body, they still possess a sense of smell. In this respect there is a difference between the anterior and posterior ends of the body, the anterior end being more sensitive. This sense is one of the factors in the burrowing of A. fœtida, contact and moisture alone being insufficient to call out the burrowing reaction.

DIRECTIVE INFLUENCE OF LIGHT.

Preliminary experiments to determine the reactions of A. fœtida to light were made in the following ways.

Worms were laid on a piece of wet filter paper lighted mainly from one side, and with screens on the other sides to cut off most of the extraneous light. Under such circumstances the animals generally crawled to the darker side of the paper. A tin box, two inches in height, two inches wide, and eighteen inches long, painted black inside and having one glass end, was next used. Wet filter paper covered the floor of the box, and the entire apparatus except the glass end, which faced a sunless window, was covered with black oilcloth. A worm was placed on the filter paper, its length across the box, and the apparatus was covered. After two minutes the cover was lifted and the position of the worm noted. Of twenty experiments made in this way, fifteen showed in general a negative reaction, that is, the worm had moved to the darker end of the box, and away from the light, while five reactions were positive. This method is objectionable practically because one cannot watch the worm, and theoretically because the narrowness of the box inevitably brings the worms against one side, and introduces a new factor, thigmotaxis. The apparatus was therefore abandoned.

In these preliminary experiments the most marked features were the absence of regularity of response and a certain individual difference in reaction. Therefore I isolated fifteen large, normal, apparently healthy worms, keeping each in its own dish of manure, and distinguishing them by letters. An attempt was made to have this number include two series of worms, one sexually active and the other not active, using the swollen clitellum as the criterion. But this phase appears so suddenly and passes so quickly in individual worms, that it was found impossible to conduct long series of experiments on worms in the sexual condition. Three sets of preliminary experiments were made on each of these fifteen worms. A sheet of wet gray filter paper eighteen inches square was placed on black oilcloth, giving a dark surface. The oilcloth lay on a table in front of a sunless window, and light from the other sides was carefully cut off by black screens. The worm to be experimented on was taken from the manure with as little disturbance as possible, an ordinary flexible section lifter serving the purpose better than forceps, and laid carefully on the filter paper. It was placed in the middle so that the head and more than half the length of the body were either (a) across the paper parallel to the surface of the window, or (b) pointing toward the window, or (c) pointing away from the window. In these three sets there was no attempt to make a definite number of experiments on each of the three directions, but we endeavored to see how the worms behaved when headed once and then left free to crawl. A "set" refers to all the movements made by one worm during one experiment, and not to a succession of experiments in which the worm was headed the same way each time. Each worm was allowed to crawl freely for ten minutes in Set 1, and for five minutes in Sets 2 and 3, and the results recorded by drawing the course of the worm on a reduced plan of the apparatus, with marginal notes where they were necessary to explain the sketches. Whenever the worm entirely left the filter paper, it was replaced carefully, and this new position indicated as the beginning of the second, third, or fourth advance in that set, by the appropriate numeral. The worm was usually replaced in or near the centre of the plate, in approximately the same relative position in which it had just crawled away; that is, if a worm crawled over the back edge, it was replaced in the centre with the head toward the back. If in crawling the worm partially left the paper, then returned tail first and assumed a new direction by turning sharply aside, that position was similarly indicated as the beginning of an advance.

Under such circumstances worms differ greatly in their manner of crawling. Some move deliberately, with much testing of the regions around the head. Others crawl quickly and "impetuously," leaving the wet filter paper for the dry oilcloth without seeming to appreciate the difference in surface, although the oilcloth soon dries their skins. Some begin to crawl rapidly the instant they are placed on the filter paper, but soon reduce their speed; others contract when first put down, and are quiet for some seconds (15–60) before crawling, but when they move, turn at once in a definite direction and crawl with increasing speed in a straight line. Most of the worms move the head in various directions before beginning to crawl, as if exploring and "testing" the neighborhood, and it was not at all common for the first movement of the head to be in the direction in which the worm ultimately crawled. With such differences in habit it follows that there is a varying number of advances for each worm, for the duration of the experiment, and that the records are widely different. Four entire records are given, which represent the extreme variations in response.

Worm C. Set 2. March 8, 1901. Sluggish individual. When put down, contracted, lifted head slightly, moved head against paper and was still sixty seconds. Turned head, crawled slowly with many stops toward back; moved more quickly when body was straight. At edge of paper it paused, explored, half left paper and withdrew to it; turned on itself and crawled toward front across paper. Half-way across it turned to left, crawled to edge of paper, withdrew once from oilcloth, then crawled off toward back on oilcloth. It withdrew tail first to filter paper, turned and crawled toward back.

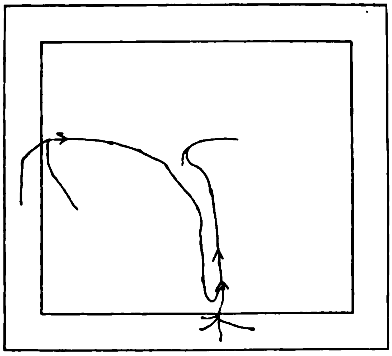


FIGURE 2. — Worm C, Set 2.

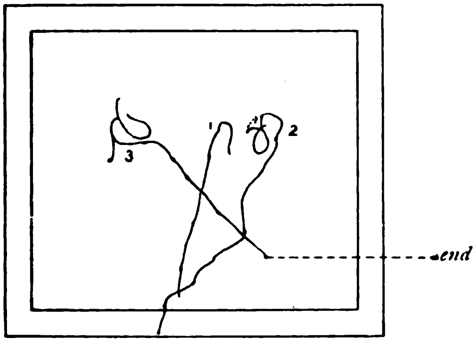


FIGURE 3. — Worm K, Set 2.

Worm K. Set 2. March 9, 1901. Worm turned and crawled at once, going quickly over back edge. When replaced at 2, turned its head to the left and remained quiet ten seconds; turned toward back, crawled (more slowly than before) in diagonal line and went over edge. Replaced at 3, turned head at once but slowly toward back and crawled diagonally back.

Worm O. Set 2. March 9, 1901. Worm quiet twenty seconds, then crawled quickly forward diagonally to right. Left paper without recoil, crawled more slowly on the oilcloth, moving toward front and then to left, and finally crawled on the filter paper again. Crossed filter paper and left it by left back corner. Replaced at 2, worm coiled, crawled toward front, left paper by front edge, turning slightly to right on the wood.

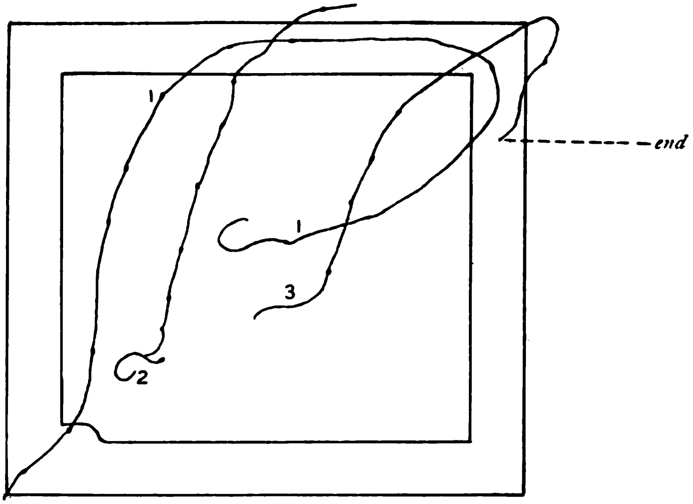


FIGURE 4. — Worm O, Set 2.

Replaced at 3 it crawled diagonally to front, crawled out on wood, keeping tail on filter paper. After twenty seconds it withdrew, crawled toward back on narrow strip of exposed oilcloth.

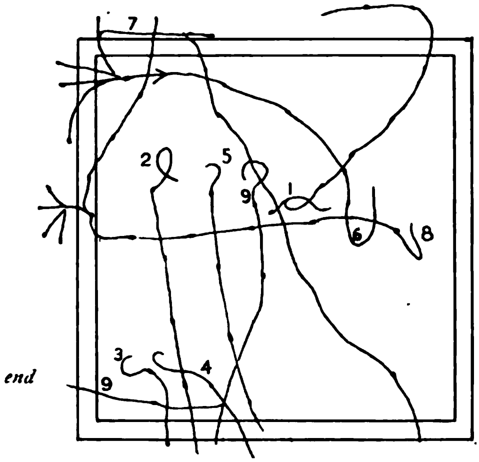


FIGURE 5. — Worm H, Set 1.

Worm H. Set 1. March 7, 1901. Worm moved head at once lifting fifteen segments; crawled quickly to right front corner, and left filter paper for wood without recoil. Replaced at 2, it crawled straight toward back and over edge with one recoil. Replaced at 3 and at 4, it went over the back edge at once; replaced at 5, crawled to back edge, recoiled from wood, turned to left and crawled over left edge without recoil. Replaced at 6, it was quiet ten seconds, crawled quickly toward front, bending to left, and at left front corner touched wood and recoiled four times. Crawled backward, turned to right and moved slowly along front edge of paper (= beginning of advance 7). Short pause, then it crawled diagonally across paper to right back and left plate without recoil. (Its course was deflected near centre of plate by small bit of manure.) Replaced at 8, worm crawled straight forward across plate, explored wood and recoiled four times, turned toward front and over edge after three recoils.

The results of the preliminary experiments are given in Table IV.

From the detailed results quoted, and from Table IV, the following conclusions are apparent.

1. The position in which the worm is placed on the paper is a factor in determining the direction in which the worm crawls. Thus the table shows that 79 per cent of the advances are positive when the worms are headed toward the window (col. 2), while when started away from the window (col. 3), 82 per cent are negative. When the worms are placed parallel to the surface of the window (col. 1) 17 per cent of the movements are indifferent (*i. e.*, directly forward) as compared with 2 per cent indifferent from the positive position and none from the negative position (col. 3).

2. When placed in the indifferent position, the worms are more likely to crawl away from the window than toward it. Thus of fifty-nine responses in column 1, thirty-six (61 per cent) are in a generally negative direction, thirteen (22 per cent) are generally positive and ten (17 per cent) are indifferent. The negative movement includes more than half the entire number in the first column, and, speaking generally, A. fœtida reacts negatively to light stimuli under these conditions.

3. When A. fœtida reacts to light it is more likely to crawl in a diagonal than in a straight or direct path. If crawling positively it is more likely to crawl diagonally than directly. A series of "diagonal" paths necessarily includes many different angles, and theoretically the line of a diagonal advance may lie anywhere between the indifferent path, parallel to the surface of the window, and the exactly negative or positive path at right angles to the former. As a matter of fact, the diagonal advances do lie at almost every angle.

4. Although *in general* A. fœtida exhibits under these conditions a negative response, there is neither constancy nor regularity in the reaction, and it is impossible to predict with certainty whether a given worm placed on the paper in the indifferent position will crawl negatively, positively, or indifferently.

The great irregularity of response in the preliminary experiments

TABLE IV.[1]

Preliminary experiments on lettered worms. Sets 1, 2, 3.

Worm.	Worm headed across plate (‖ to window).	Worm headed +.	Worm headed −.
A	−, ‖−, ‖	‖+	
B	‖+, ‖−; +, ‖−	‖+; ‖+, ‖+,‖+	−; −
C	−; −	+; ‖−	−
D	‖; +; ‖−, ‖−	+; ‖+	‖−
E	+, ‖, ‖+, ‖−; ‖−; ‖−, ‖−, ‖+	‖; +	‖+, −, −; +
F	‖, ‖+, −; ‖−, ‖+; ‖−	‖+, ‖+; ‖−; +	−
G	−, ‖−, ‖−; ‖+; ‖−	‖+; +; +	−
H	‖+; −, ‖−	‖+, ‖+	−, −, −, −, ‖−
I	‖−; ‖−	+; +; ‖+	−; −, −
J	‖−, ‖−; ‖−; −	‖; ‖+	−; −
K	‖	‖+; −, −, −; +	
L	‖−; ‖; ‖−, ‖, ‖	‖+; ‖+	
M	‖−, ‖	−; −	
N	+, −, −; ‖; −, −	+; +	−
O	‖+, ‖−, −, ‖+	‖+, +, +, ‖+, ‖+, ‖+, +	‖+, ‖+
	+ = 4 ‖− = 24 − = 12 ‖+ = 9 ‖ = 10 Generally − = 36 = 61% Generally + = 13 = 22% Indifferent 10 = 17% 59 = 100%	+ = 14 ‖− = 2 − = 5 ‖+ = 20 ‖ = 2 Generally + = 34 = 79% Generally − = 7 = 16% Indifferent 2 = 5% 43 = 100%	+ = 1 ‖− = 2 − = 17 ‖+ = 3 ‖ = 0 Generally − = 19 = 82% Generally + = 4 = 17% 23 = 99%

SUMMARY OF ALL REACTIONS IN PRELIMINARY EXPERIMENTS.

Generally − = (36 + 7 + 19) = 62 = 50%
Generally + = (13 + 34 + 4) = 51 = 41%
Indifferent (‖) = (10 + 2 + 0) = 12 = 9%
125 = 100%

---

[1] In this and all following tables of light reactions, the same abbreviations are used. + indicates a movement directly toward the source of light; − a movement directly away from the source of light; ‖ a movement parallel to the surface of the window; ‖+ a diagonally positive movement to right or left; ‖− a diagonally negative movement to the right or left.

led to the adoption of the following apparatus (see Fig. 6). A tin box, eight inches wide, four inches deep, and twelve inches long, which was painted black inside and had one end of glass, was faced against a sunless window opposite which were no buildings. The floor of the box was covered with wet gray filter paper. On this were placed thick microscopic glass slides arranged to form two narrow paths of the same width as a worm's body, one running parallel to the glass end, the other at right angles to the first and communicating with it. The slides were almost or quite as thick as the diameter of the worm, and the paths were widened or narrowed according to the size of each individual worm. The slides were then covered with a glass plate, which completed the fourth side of the paths. This device provided narrow moist passages, and had the further advantage of cutting off currents of air, and preventing undue drying. The method of using it was as follows. A worm was taken up with the section-lifter, and placed carefully on the filter paper with the head end lying in the indifferent path (||). The glass cover was quickly lowered with as little shaking as possible, and a thick black cover was drawn over the head of the observer and over the entire apparatus except the glass end. Light was thus cut off except from the front. Preliminary experiments showed that illumination of the right side of the worm produces the same effect as illumination of the left. For convenience' sake, therefore, the worms were introduced from the right, and consequently illuminated from the right side except in Set 8. A worm introduced into a narrow chink like the paths in this apparatus is almost certain to crawl forward. It is obvious that when the worm reaches the end of the indifferent path it is forced to crawl either directly front[1] or directly back, and a record of the path "chosen" was made simply as + or —. The worm was allowed to crawl entirely out of the indifferent path before the record was made. Five experiments (that is, five choices) were made in a set, that number being selected to avoid the danger

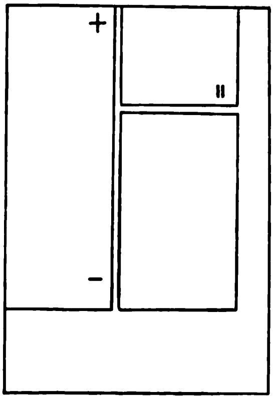


FIGURE 6.

---

[1] The words "front" and "back" refer always to the light and dark ends of the box respectively, while "forward" and "backward" refer to the manner of crawling, head first or tail first respectively.

TABLE IV.[1]

Preliminary experiments on lettered worms. Sets 1,

Worm.	Worm headed across plate (‖ to window).	Worm headed +.	W
A	–, ‖–, ‖	‖+	
B	‖+, ‖–; +, ‖–	‖+; ‖+, ‖+, ‖+	–;
C	–; –	+; ‖–	–
D	‖; +; ‖–, ‖–	+; ‖+	‖–
E	+, ‖, ‖+, ‖–; ‖–; ‖–, ‖–, ‖+	‖; +	‖+,
F	‖, ‖+, –; ‖–, ‖+; ‖–	‖+, ‖+; ‖–; +	–
G	–, ‖–, ‖–; ‖+; ‖–	‖+; +; +	–
H	‖+; –, ‖–	‖+, ‖+	–,
I	‖–; ‖–	+; +; ‖+	–;
J	‖–, ‖–; ‖–; –	‖; ‖+	–;
K	‖	‖+; –, –, –; +	
L	‖–; ‖; ‖–, ‖, ‖	‖+; ‖+	
M	‖–, ‖	–; –	
N	+, –, –; ‖; –, –	+; +	–
O	‖+, ‖–, –, ‖+	‖+, +, +, ‖+, ‖+, ‖+, +	‖+,
	+ = 4 ‖– = 24 – = 12 ‖+ = 9 ‖ = 10 Generally – = 36 = 61% Generally + = 13 = 22% Indifferent 10 = 17% 59 = 100%	+ = 14 ‖– = 2 – = 5 ‖+ = 20 ‖ = 2 Generally + = 34 = 79% Generally – = 7 = 16% Indifferent 2 = 5% 100%	

SUMMARY OF

50%

41%

9%

100

same

face of

mally

[illegible]

K	0	0	1	0	4	0	0	1	2	[illegible]																				
L	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0											
M	0	0	0	1	4	0	1	0	0	4	0	0	0	3	2	0	0	2	0	3	0	1	1	1	2	0	2	3	5	15
N	0	0	1	0	4	0	0	2	0	3	0	0	1	0	4	0	0	3	0	2						0	0	7	0	13
O	0	0	1	1	3	0	1	0	0	4	0	0	2	0	3	0	0	0	0	5	0	1	2	1	1	0	2	5	2	16
Totals	0	0	15	3	52	0	2	13	3	47	0	0	14	3	48	0	0	13	6	46	0	5	13	5	37	0	7	68	20	230

length. But when it reached the other end of that path it was free to crawl in any direction. The great number of possible directions were grouped under five divisions, the same as those used above in describing the preliminary experiments. Fig. 7 shows the range of each of these signs. If the worm as it left the indifferent path turned at right angles to that path, the reaction was recorded as a simple plus or minus, whether the turn was made as soon as the worm issued from the path (and consequently when the worm continued to crawl against a glass side), or at some little distance from that path. Experience showed that such a sharp turn was always made at or near the end of the indifferent path. With the exception of Set 13, the record was made in each case simply by the use of the appropriate sign, after the worm had entirely crawled out of the indifferent path. The results of Set 13 were recorded differently (in a manner to be described below), although the same signs were used. As in the preceding group, all starts were made from the right except in Set 9. The results of the experiments with this apparatus are given in Table VII.

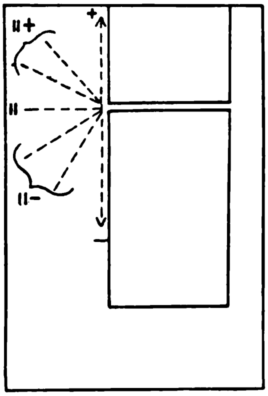


FIGURE 7.

It is to be noticed first that out of three hundred and twenty-five experiments, none are directly plus, and only seven are directly minus. Sixty-eight advances (21 per cent) are continuations of the indifferent path, a high proportion when one considers the tendency of this species to be deflected from a straight course by objects with which it comes in contact.[1] Twenty (6 per cent) are diagonally plus, while a majority of all the reactions are diagonally minus, — two hundred and thirty out of three hundred and twenty-five (71 per cent). Adding the directly negative and diagonally negative, there is a total of two hundred and thirty-seven generally negative reactions, against twenty in a generally positive direction. It thus appears that under these conditions, with the course not determined, A. fœtida has a negative reaction.

It is to be particularly noticed, however, that of the two hundred and thirty-seven negative responses only seven are directly away from

---

[1] See section on Thigmotaxis.

TABLE VII.

Worm.	SET 5.					SET 7.					SET 9.					SET 11.					SET 13.					SUMMARY.				
	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−	+	−	‖	‖+	‖−
A	0	0	1	0	4	0	0	1	0	4	0	0	2	0	3	0	0	1	2	2	0	0	1	0	4	0	0	6	2	17
B	0	0	2	1	2																					0	0	2	1	2
C	0	0	3	0	2	0	0	2	0	3	0	0	0	0	5	0	0	2	0	3	0	0	0	0	5	0	0	7	0	18
D	0	0	2	0	3	0	0	3	0	2	0	0	3	0	2	0	0	0	2	3	0	0	4	0	1	0	0	12	2	11
E																														
F	0	0	2	0	3	0	0	2	0	3	0	0	0	0	5	0	0	2	0	3	0	0	2	0	3	0	0	8	0	17
G	0	0	1	0	4	0	0	0	0	5	0	0	2	0	3	0	0	0	0	5	0	1	1	0	3	0	1	4	0	20
H	0	0	0	0	5	0	0	0	0	5	0	0	1	0	4	0	0	1	1	3	0	0	0	0	5	0	0	2	1	22
I	0	0	0	0	5	0	0	0	0	5	0	0	2	0	3	0	0	1	0	4	0	0	0	3	2	0	0	3	3	19
J	0	0	1	0	4	0	0	2	1	2	0	0	0	0	5	0	0	1	1	3	0	1	0	0	4	0	1	4	2	18
K	0	0	1	0	4	0	0	1	2	2	0	0	1	0	4	0	0	0	0	5	0	0	1	0	4	0	0	4	2	19
L	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	1	1	0	3	0	1	1	0	23
M	0	0	0	1	4	0	1	0	0	4	0	0	0	3	2	0	0	2	0	3	0	1	1	1	2	0	2	3	5	15
N	0	0	1	0	4	0	0	2	0	3	0	0	1	0	4	0	0	3	0	2						0	0	7	0	13
O	0	0	1	1	3	0	1	0	0	4	0	0	2	0	3	0	0	0	0	5	0	1	2	1	1	0	2	5	2	16
Totals	0	0	15	3	52	0	2	13	3	47	0	0	14	3	48	0	0	13	6	46	0	5	13	5	37	0	7	68	20	230

the light, the remaining two hundred and thirty being more or less diagonal. The position, *i.e.*, the angle of these diagonal responses varied so much that in recording Set 13 a new method was adopted. The direction of the worm, as it issued from the indifferent path and

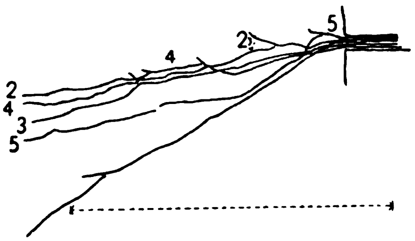


FIGURE 8. — Worm D, Set 13.

Record:

1. ||– 2. || 3. || 4. || 5. ||

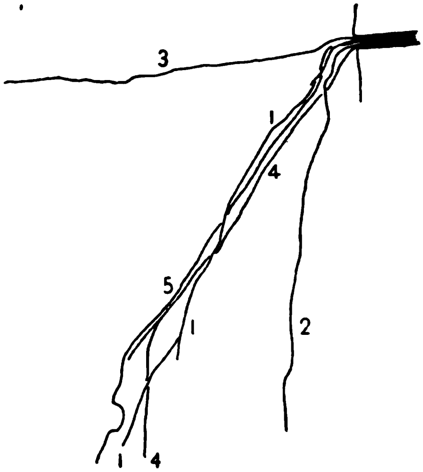


FIGURE 9. — Worm G, Set 13.

Record:

1. ||– 2. – 3. || 4. ||– 5. ||–

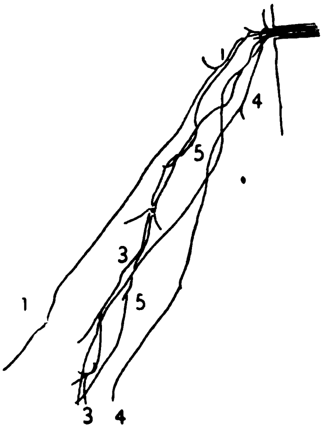


FIGURE 10. — Worm H, Set 13.

Record:

1. ||– 2. ||– 3. ||– 4. ||– 5. ||–

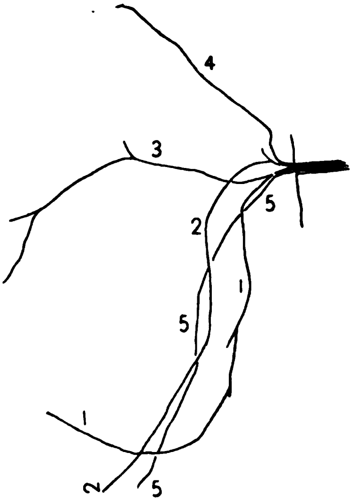


FIGURE 11. — Worm M, Set 13.

Record:

1. – 2. ||– 3. || 4. ||+ 5. ||–

travelled as far as the length of its own body, was traced directly on the plate forming the roof of the apparatus with a brush dipped in India ink. It was then drawn on tracing linen. Figures 8, 9, 10, and 11 are typical examples of the results obtained by this method. The end of the indifferent path is indicated by the straight lines; for the sake of clearness the path is widened in the diagram. Only the long axis of the worm is shown, so that it is possible to include in one diagram the five advances belonging to a set. The twenty experiments shown were made in the same day within one hour, and under the same conditions. They were selected, because they show the extreme differences. In Fig. 8 all five paths lie close together, mainly in the indifferent direction. In Fig. 9 they are more scattered, one indifferent, one directly negative, and three diagonally negative. In Fig. 10 they are close, but at a different angle from those in Fig. 8, while in Fig. 11 they are widely scattered.

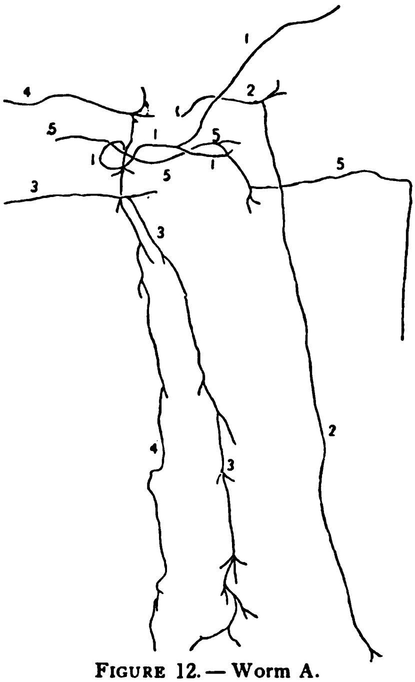


FIGURE 12. — Worm A.

A third series of experiments was made on these fifteen worms, in which the apparatus on the floor of the box consisted only of a glass plate supported at such a height that it just touched the back of the worm. The worm was laid on the filter paper in the indifferent position, the cover quickly and carefully lowered, and the course traced as before. Some of the results are given in Figs. 12, 13, and 14. They show the same variety of response that was obtained in all the preceding experiments. Incidentally these tracings also show the amount of feeling and testing of the surrounding region that precedes most forward crawling.

This great variation can mean only one of two things. Either, under the conditions of the experiments, light has so little directive effect that minor influences such as slight irregularities in the surface of the paper suffice to turn the animals away from an ideal course leading directly from the light; or Allolobophora fœtida does not respond to light stimuli with the mechanical precision predicated for other earthworms. The filter paper used possessed a slight grain, and to avoid having a surface with constant marks and ridges, the pieces were cut in varying directions across or with the grain. No difference was observed in the results. The illumination was constant through each set of experiments, while the apparatus cut off air currents and provided equal contact on all sides of the body. The conditions therefore were constant, yet the reactions varied considerably. The more carefully the habit of crawling of this species was watched, the more I inclined to the second conclusion, that A. fœtida, while it has a negative reaction to diffuse daylight, does not naturally respond sharply and with mechanical precision. The orientation of the body in relation to light, which theoretically would bring the long axis in a directly minus-plus path (*i. e.*, in a path parallel to the light rays and with the head pointing away from the source of light), is inexact and poorly defined unless the worm is forced to crawl in the directly minus-plus path.

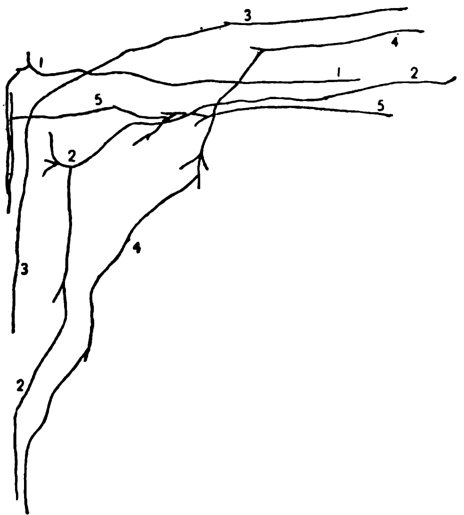


FIGURE 13. — Worm H.

It may be worth while to consider here the factors other than light that could enter into the problem. The grain of the filter paper, mentioned above, was a constant factor through any given set, yet results varied. Therefore the irregularity of response can hardly be due to the grain of the filter paper.

It occurred to me that the previous path may exert some influence, that is, it is possible that an actual trace is left by the worm in crawling, which guides the animal the next time. This I do not think a factor of any importance, for the following reasons. First, there was no indication that the first reaction determined the second, the second the third, and so on, which would necessarily be the case if the previous path exerted an influence. On the contrary, each successive path varied without relation to the succeeding paths. Second, the filter paper was renewed after each set of five, so that the paths of one set could not have affected those of the following sets. Third, in some previous experiments (not included in the above report), in which fresh paper was used for each single reaction, no difference was observed in the results as compared with those in which the filter paper was less frequently changed.

The influence of the direction from which the worm starts, noted in the preliminary experiments, is also apparent in the results of Series 3. When one considers the marked thigmotaxis of A. fœtida, sixty-eight indifferent movements out of three hundred and twenty-five (21 per cent) seems a large proportion, and is only to be accounted for by the tendency of the animal to continue crawling in the same direction. It is worth noticing that if the worm crawled rapidly through the indifferent path, it was more likely to continue in that direction without turning than when it crawled slowly and deliberately. If the speed at which the worm traverses the indifferent path is an important factor in modifying the effects of light stimuli, then the slower the movement of the worm through the indifferent path, the more decidedly negative should be the response when it leaves that path; while those moving rapidly through the indifferent path ought to show a less negative response. To determine this point exactly would involve the use of apparatus to measure the rates of speed in each experiment, and this is yet to be done.

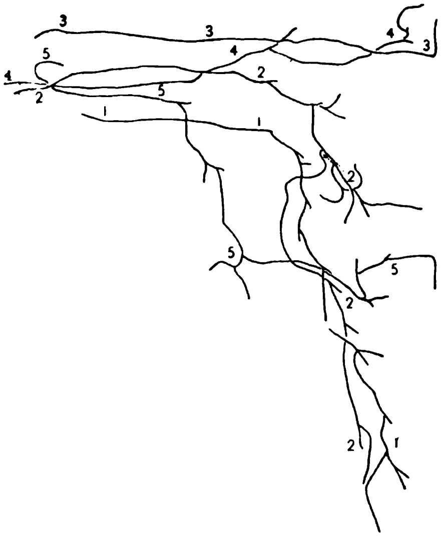


FIGURE 14. — Worm I.

There is some slight indication of consistent individual differences, not bound up with the sexual phase, for an examination of Table V shows worm J made four of the seventeen plus reactions in the whole series. The records of the preliminary experiments also showed this in the terms used to describe the worms. One individual was described in each set as "sluggish," the recorder not noticing until the end of the series that the word had been used before, while others were twice described as "lively, irritable" worms. However, the individuality is slight and not sharply defined, although it undoubtedly exists.

After the experiments above recorded were completed, a paper appeared by Prof. G. H. Parker and Mr. L. Arkin in this Journal for April, 1901. The authors find that "The earthworm Allolobophora fœtida (Sav.) when exposed to light of moderate intensity creeps away from the source of light, *i. e.*, is negatively phototactic," and that 26 per cent of head movements were away from the light when the entire animal was illuminated from one side. With the general statement that the animals are negatively phototactic my results are fully in accord. I found, however, that when the worms were free to crawl in any direction after traversing a path in which they were illuminated from one side by diffuse daylight, the negative percentages were higher, and when forced to crawl in one of two definite paths were much higher than in the results quoted from Professor Parker and Mr. Arkin.

THIGMOTACTIC REACTIONS, AND THE MODIFICATION OF THIS RESPONSE BY LIGHT.

It is a common observation that earthworms have the habit of lying coiled together in masses. Darwin[1] refers to this habit of Lumbricus in the "Formation of Vegetable Mould" and Hoffmeister[2] says earthworms pass the winter either singly or rolled up with others at the bottoms of their burrows. Allolobophora fœtida has a similar habit. When a number of these worms are kept in a glass dish they can be seen lying against the bottom either coiled together or applied against each other in straight lines. Often the entire mass of worms is found lying in the angle formed by the bottom and sides of the dish. If two or more worms crawling on wet filter paper touch each other, they place themselves side by side. Sometimes they cling so closely in this position that it is difficult to separate them. If a worm in crawling turns so that its head touches its body, the worm turns on itself and crawls along its own body until the head projects beyond the tail, when it may either continue crawling forward, straightening out the loop, or, turning again on itself, it may bury the head beneath the other folds. If there is an object on the filter paper making an angle with it, the worms crawl into the angle if they touch the object, and if the latter is dark the worms will not leave it for light areas.

---

[1] DARWIN: Formation of Vegetable Mould by the Action of Earthworms, p. 34.

[2] HOFFMEISTER: Familie der Regenwürmer, p. 13.

Pieces of cylindrical glass rod one quarter of an inch in diameter, of varying lengths in comparison with the length of the worm, were laid in pairs on the filter paper in contact with each other so that a triangular passage was formed between them and the filter paper. Since the rods were transparent, the artificial burrow thus provided was little darker than the paper outside. The worm was laid down near one end of this small passage so that in crawling the head came in contact with the ends of the rods. Such a contact resulted always in a series of investigating head-movements, in the course of which the passage was found. As soon as the head happened to be poked into the narrow chink the worm crept forward into it, often quite rapidly. If the rods were longer than the worm it remained in the passage for some time (varying from eight to twenty minutes), but sooner or later it crawled entirely through and out the opposite end, usually after one or two recoils. If the rods were shorter than the worm the animals entered as before, but either crawled straight through or remained a short time (one to two minutes). If rods are very gently laid on each side of the head while the worm is extended upon the filter paper, so that the head lies within the channel, the worm usually crawls forward, but if the rods are similarly laid against the tail there is no backward crawling into the passage. In those cases where a worm remained in a passage shorter than the body it necessarily happened that a part of the body must lie outside. This uncovered portion was always the tail end. That is, the worms remained quiet for a short time with the tail protruding, but as soon as they crawled far enough forward to thrust out the head they left the passage.

A series of experiments was next made with the apparatus of which a ground plan is shown in Fig. 15. Three thick microscopic glass sides were placed end to end across a square of wet filter paper so that they formed a transparent barrier (*A*, Fig. 15) parallel to the surface of the window by which the apparatus was lighted. A narrow black box (*B*) standing on the slides cast a strip of shadow (*D*) on the filter paper, while between the slides and this shadow was a narrow strip of light (*C*). Light from the other sides was cut off by black screens. Experiments were made in diffuse light and in sunlight.

Diffuse Light. — Worms put down in the strip of shadow explored the lighter areas on each side before stretching the head far enough to touch the glass. As soon as the head touched the slides the worms crawled against the glass, thereby lying directly in the light. In this position they remained from one to five minutes, making many exploring head movements and crawling back and forth along the side of the glass. They did not crawl around the free ends of the slides (in which position they would have pointed directly toward the window), but recoiled when the head extended beyond the end. The worms finally left the glass, not by turning the head away from the light and crawling away in a definite direction, but on the contrary this occurred only when the worm in exploring moved the anterior third of the body away from the glass. When this portion of the body was no longer in contact with the glass, the worm crawled away across the light paper, usually in a more or less negative direction. The entire apparatus was now shaded so that there was no difference in illumination between different areas immediately behind the glass, although the remainder of the paper was brightly illuminated. The worms were placed so that in crawling they soon came against the slides. The animals now crawled against the slides as soon as the head came in contact, but instead of crawling away after a short time remained as long as the slides were entirely shaded (ten minutes). During this time the worms explored as before, recoiling each time the head was thrust into the light. When the cover was removed and the apparatus illuminated as in the preceding experi-

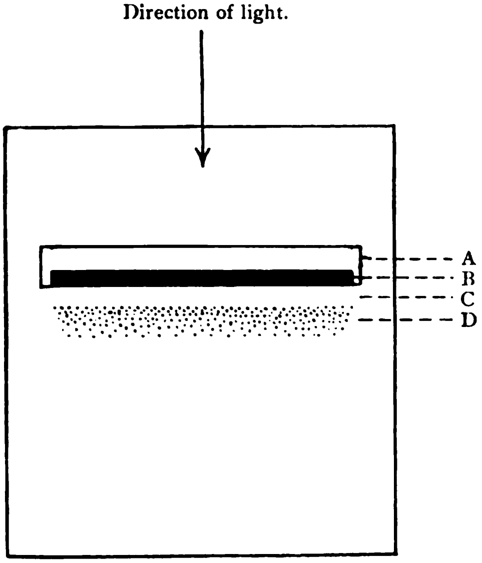


Figure 15.

ments the worms crawled back and forth, explored, and soon deserting the strips, moved across the filter paper.

These experiments were repeated on worms from which ten or twelve anterior segments had been cut. When put down on the filter paper with the anterior end in contact with the slides, the animals crawled away tail first. If the barrier was laid across the path of a forward-moving mutilated worm, the animal crawled backward for a short distance after touching the barrier, and sometimes repeated this several times before showing any disposition to apply itself against the barrier. If it did come to lie against the strips there was no testing of the adjacent regions as in normal worms, but on the contrary the worms crawled backward and forward along the glass and finally left it in an entirely indefinite manner.

Sunlight. — When the same apparatus was illuminated by direct sunlight and normal worms placed in the strip of shadow, *D*, they tested the areas on each side of the shadow and crawled quickly over the sunlit paper, whether the head had touched the slides or not. If placed against the slides in the light strip, there was usually a short latent period (ten to twenty seconds), and then the worm crawled away as before. If put on the paper in front of the slides and started crawling toward them the worms tried at first to push through, but failing in that they turned along the glass, crawled to the end and either continued moving in a straight line or turned around the end of the glass.

Beheaded worms exposed to full sunlight crawled at once in the direction in which the anterior end happened to point. If they came against the slides they showed no tendency to remain there.

The next apparatus consisted of a small glass box (⅝ in. × 3¼ in. × 3¼ in.) open on one of the four small sides and resting on wet filter paper on one square side. By means of microscopic slides the level of the paper outside was raised flush with the floor of the box. The opening was turned either toward the window, away from the window, or toward one side. The apparatus was illuminated from one side with diffuse light from a sunless window, as above. When a worm crawling over the wet filter paper touched the outside of the box, it turned, applied itself against the box and followed the outline of the latter. Upon reaching the open side the worm crawled around the edge and into the box, keeping always in the angle formed by the bottom and side. It then travelled around the three sides, sometimes doubling on itself and sometimes without interruption of its forward movement. If the box was now entirely shaded the worm remained in it for some time (half an hour to two hours). If partially shaded it coiled against the side within the shaded portion; if left unshaded it crawled out of the box at one corner. When the head of the worm entered the open side of the box at any point too far away from a corner to have more than the ventral surface in contact with the glass, the worm withdrew from the box and turned in another direction. In general, Allolobophora does not leave wet filter paper for glass, and this experiment shows that it is not contact with glass as a substance that causes entrance into the box, but contact on more than one surface of the worm's body.

The box was next placed on the filter paper in an almost vertical position, with the open end down and resting on one long edge so that a worm could crawl under the open end. The worms were then made to crawl against the inner side of the supporting edge, near one angle. Out of six experiments only one worm entered entirely. It crawled around the three sides, remaining quiet against the top for three minutes, then down a vertical side and out. The others tested the vertical angles and one entered for a third of its length, but all withdrew and crawled away. When the box in this position was covered with black paper two worms entered it and were found against the top. A third did not really enter, but slipped between the box and its paper covering, without vertical crawling. When the uncovered box was supported vertically with the open side up and at the level of the plate on which the filter paper rested, the worms turned down into it as soon as the head ran over the edge of the plate, so as to project over the open side of the box, crawled down one side, around the bottom and finally to the open top after a shorter or longer pause.

By means of eight narrow strips of glass imbedded in paraffin on the oblong bottom of this box, the interior was subdivided into nine narrow spaces. It was thus almost impossible for the worm to explore the open end of the box without finding one or more corners. In addition to the numerous corners and angles thus provided, there were many small splits in the paraffin, while the latter contracted entirely away from the glass in the centre of each long side, leaving a narrow crevice which communicated with each of the nine narrow subdivisions. When the box stood vertically open end up and at the level of the plate, the worms crawled in, down one corner to the bottom and after much exploring wedged themselves into the darker of the two narrow crevices mentioned above, between the paraffin and the box. If the box was turned over so that the worms came to lie on the lighter side, there was at first no response, but after one or two minutes the worms moved slowly until the head was thrust into shadow, either of its own body or of the paraffin.

When the box with its partitions was placed on the filter paper on one square side, with the opening away from the window, the worms entered as soon as the head touched any partition, and crawled to the back. In all cases the worms crawled as far as possible *under* the paraffin (even though they thereby left a comparatively dark shadow cast by the paraffin for a lighter strip). If the box was turned over, so that the worm was on top of the paraffin and in full light, it slowly changed its position so that it lay *under* the paraffin again. If left uncovered, the worms crawled out of this box after from half an hour to an hour. If covered with black oilcloth and the filter paper kept constantly moist they remained in it for a long time. One individual remained for over eighty hours, apparently moving but little through the chinks and crannies at the back of the box except when the cover was lifted. Then it crawled quickly up and down the angles, but did not leave the box. If a worm is laid on wet filter paper covered darkly with oilcloth and left for some hours, it does not remain on the dark wet filter paper, but is found dried up at some distance. The wet filter paper surely offers a surface more natural than the glass box, even when this is sprinkled inside. The fact that the worms remain in the box so long a time is therefore undoubtedly due to thigmotaxis.

Summary. — Allolobophora fœtida possesses a marked thigmotactic response. In diffuse light the response to contact with a transparent object (such as the glass barrier) overcomes for a time the restlessness and negative reaction to light. In direct sunlight the restlessness is so extreme that it entirely overpowers the thigmotactic response. Sooner or later the thigmotactic reaction is overcome by light, the time during which light must act bearing an inverse relation to its intensity. The smaller the crack, provided the worm is able to enter it, the more marked is the response when the worm pokes its head into one end, and consequently the stronger must be the light stimulus to overpower thigmotaxis. In the dark, thigmotactic response to contact on all sides of the body, such as is offered by a narrow passage, is so strong that the worm remains for days in a somewhat dry and certainly unnatural environment.

This work was done in the Biological Laboratory of Bryn Mawr College, under the guidance of Professor Thos. H. Morgan, and I wish to take this opportunity of thanking him for his criticism and advice.

LIST OF REFERENCES.

DARWIN, C. : The Formation of Vegetable Mould through the Action of Worms, with Observations on their Habits.
FOOTE, KATHARINE : Journal of morphology, 1898, xiv, pp. 481–506.
GRABER : Grundlinien zur Erforschung des Helligkeits- und Farbensinnes der Thiere. Leipzig, 1884.
HESSE, R. : Zeitschrift für wissenschaftliche Zoologie, 1896, lxi, pp. 393–419.
HOFFMEISTER : Die bis jetzt bekannten Arten aus der Familie der Regenwürmer.
LOEB, JACQUES : Der Heliotropismus der Thiere, und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen. Würzburg, 1890.
PARKER and ARKIN : American journal of physiology, 1901, v, p. 151.
WILSON, EDMUND B. : Journal of morphology, 1889, iii, pp. 388–462.

Further Experiments on the Regeneration of Tubularia.

By

T. H. Morgan.

With 25 figures in text.

Eingegangen am 17. December 1901.

The work of Bickford, Driesch, and myself has shown that small pieces of the stem of Tubularia often produce parts of whole hydranths instead of reduced hydranths of proportionate size. Driesch discovered that the more distal parts of the stem give rise more often to these incomplete structures. My own work lead me to infer that also other factors, than that of nearness to the end are involved in the regeneration, and I suggested that the behaviour of individual stems, and of different regions of the same stem might possibly be due to the age of the stem in the sense that recently formed stems are more likely to give rise to incomplete structures than stems that have been formed for some time. The practical difficulties of testing this view are not perhaps very great, since this only involves keeping newly formed individuals alive for some time, but the colonies that I kept in aquaria during the past summer at Woods Holl, did not grow sufficiently well and the new hydranths did not remain alive long enough to make the results a success. Moreover during July and August the colonies of *Tubularia* (*Parypha*) *crocea* were beginning to disappear in their natural haunts, and by the end of August had almost completely disappeared. Nevertheless by selecting the most vigorous colonies, and those which had obviously been recently growing and forming new shoots I obtained enough material to give some results of interest. My main effort was directed towards observing the behaviour of consecutive pieces from the distal end of the same stem, and comparing these results with those from similar pieces of varying lengths from other stems. The method previously most often employed by Driesch and by myself of recording the results from pieces having the same numerical position in different stems is less exact for the purposes that I had in view[1].

A preliminary examination of the regeneration of pieces of the American form (*T. crocea*) showed that short pieces give the same incomplete structures and double structures that are found in the Neapolitan form as observed by Driesch and by myself, and in *T. tenella* studied by Bickford. In describing these new results I shall make use of the same nomenclature and abbreviations used in my former paper in which figures of the different forms are given. Thus, pb. stands for proboscis (Fig. *G* of my previous paper); hy. + stem stands for hydranth and stem (Fig. *A*); hy. + stalk for hydranth and stalk (Figs. *B*, *C*); hy. for hydranth (Fig. *D*); d. pb. for double proboscis (Fig. *K*); d. hy. for double hydranth (Figs. *H*, *J*) etc. In further explanation of the terms used in the tables to designate the different incomplete structure it should be pointed out that while a hydranth (hy.), a hydranth plus reproductive organs (hy. + reprod.), and a proboscis (pb.) are incomplete structures, a hydranth plus stem (hy. + stem) is not an incomplete structure, and a hydranth plus stalk (hy. + stalk) is doubtfully an incomplete structure, and one only in the sense that proportionately more of thè piece has been used in forming the hydranth than in forming the stalk. Even the hydranth (hy.) in these tables is in itself complete, but since it lacks a stalk, or a stem it is an incomplete hydroid, but not an incomplete hydranth.

The following tables, I—IX, give the sizes of the pieces and the kinds of structures that regenerate from consecutive short pieces of the same stem. The pieces were of about the same length[2]. The first piece, 1, is in these, and in all following tables the most distal piece, and lay just behind the hydranth[3].

---

[1] Driesch gives five series of consecutive pieces from the same stem.

[2] The numbers correspond to divisions of the eye-micrometer. Thirty-two divisions equal one millimeter.

[3] The average length of normal hydranth-forming regions is about 20 divisions for pieces cut off some distance from the distal end, Fig. *L*. The length of a hydranth-forming region, nearer the old hydranth, may be double this length, Fig. *C*.

Table I.

No. of piece	Size of piece	Kind of structure
1	20 (× 8)	dead
2	22	d. pb.
3	29	d. pb.
4	21	d. pb. (?)
5	28	hy. + stalk

Table II.

No. of piece	Size of piece	Kind of structure
1	42 (× 8)	pb.
2	39	hydranth
3	38	- + stalk
4	38	- - -
5	45	- - -

Table III.

No. of piece	Size of piece	Kind of structure
1	40 (× 11)	d. pb.
2	46	d. pb. + reprod.
3	41	d. hy. + reprod.
4	45	d. pb.
5	50	hydranth

Table IV.

No. of piece	Size of piece	Kind of structure
1	60 (× 16)	d. pb.
2	62	d. pb. + reprod.
3	60	hy. + stalk
4	75	d. hydranth

Table V.

No. of piece	Size of piece	Kind of structure
1	52	d. pb.
2	50	d. pb. + reprod.
3	49	hydranth
4	50	d. pb.

Table VI.

No. of piece	Size of piece	Kind of structure
1	29 (× 15)	pb.
2	25	nothing
3	29	d. pb.
4	30	pb. + reprod.

Table VII.

No. of piece	Size of piece	Kind of structure
1	40	d. pb.
2	44	hy. + stalk
3	42	pb.
4	35	dead
5	40	d. pb. (?)

Table VIII.

No. of piece	Size of piece	Kind of structure
1	40	pb.
2	lost	lost
3	46	pb.
4	48	hy. + few tentacles
5	42	hy. + very few tent.

Table IX.

No. of piece	Size of piece	Kind of structure
1	85	d. pb. + reprod.
2	95	hydranth
3	98	-
4	98	d. hydranth
5	102	hydranth

From these tables it would be difficult to draw any positive conclusion in regard to the incomplete structures. The results are especially difficult to interpret owing to the presence of so many double structures; and we do not know what factors determine the formation of double structures in preference to single ones. Nevertheless the tables indicate, so far as they show anything definite, that there is a certain similarity in the behaviour of the pieces of the same stem, but I should hesitate to state that this is so on the insufficient evidence from these experiments alone.

There is a further point in regard to the double structures that has not been previously noticed by either DRIESCH or myself, although it was perhaps tacitly implied. The double structures always produce the same kind of complete or incomplete structures at both ends. For instance, if a double structure has a whole hydranth at one end, it has a whole hydranth at the other end; if it has a proboscis at one end, it has a proboscis at the other end, etc. There is never produced a hydranth at one end and a proboscis at the other. The meaning of this is not far to seek. The influences at the two ends are so nearly the same that the material is equally divided between the two products. If there is enough material for a whole hydranth to develop at each end then a double hydranth is produced, but if the piece is too small to produce, under the conditions, two whole structures, then two incomplete and equivalent structures develop. This conclusion fits in very well with another one that I have called attention to, viz., that in these double structures the two ends develop at exactly the same rate. This is the more surprising, because when long pieces are used the proximal hydranth is usually twenty-four hours behind the distal one in its appearance. In small pieces the entire development is retarded. It may not be going too far to assume that in these pieces the development of the distal and proximal hydranths mutually influence the rate of development of each other.

At the time of year at which I worked many of the stems were sending out at the sides a large crop of new short stalks ending in hydranths. In fact, many bunches were composed of old, sometimes headless stalks with lateral branches. I attempted to find out if short pieces from the basal ends of these new, short branches would produce whole hydranths, like those from the basal region of long pieces, or incomplete structures like those from the proximal ends of longer pieces — the two regions being, as far as distance alone is concerned, about the same. A few experiments that I made showed that incomplete structures are most often formed. This may be due either to the nearness of the piece to the original hydranth, according to Driesch's principle, or to the newness of the new stalk according to the view which I think is probably true — or, in fact, to both together. The results in fourteen cases are here given; each short piece was cut from the base of a different branch[1].

Table X.

Size of piece	Kind of structure	Size of piece	Kind of structure
15 (× 10)	d. pb.	20 (× 13)	pb. + reprod.
20	hy. + reprod.	34 (× 12)	hy. + stalk
25	- - -		
22	d. pb.	25 (× 8)	hy. + stalk
		20	pb.
22 (× 12)	hydranth	10	pb.
20 (× 11)	hy. + stem	23	hydranth
8 (× 10)	d. pb.		
18 (× 10)	pb. + reprod.		

In a few cases a variation of the first series of experiments was carried out. From the distal end of each stem a series of consecutive pieces of increasing or of decreasing lengths were cut off. The following tables XI—XV give the results.

Table XI.

No. of piece	Size of piece	Kind of structure
1	52 (×14½)	pb. + reprod. (?)
2	40	pb.
3	33	pb. + reprod.
4	30	d. pb.
5	25	d. pb. (?)
6	20	d. pb. (?)

Table XII.

No. of piece	Size of piece	Kind of structure
1	75 (× 13	d. pb. + reprod.
2	60	hy. + stalk
3	52	- - -
4	45	hydranth
5	36	hydranth

Table XIII.

No. of piece	Size of piece	Kind of structure
1	58	dead
2	41	pb. + reprod.
3	35	d. pb.
4	30	pb.
5	16	d. pb. (?)

---

[1] The brackets indicate that the pieces came from the same colony.

Table XIV.

No. of piece	Size of piece	Kind of structure
1	20 (× 11)	dead
2	29	d. pb.
3	45	d. pb. + d. reprod.
4	52	d. pb. + d. reprod.

Table XV.

No. of piece	Size of piece	Kind of structure
1	23	dead
2	29	d. pb.
3	30	d. pb. + reprod.
4	50	d. pb. + reprod.

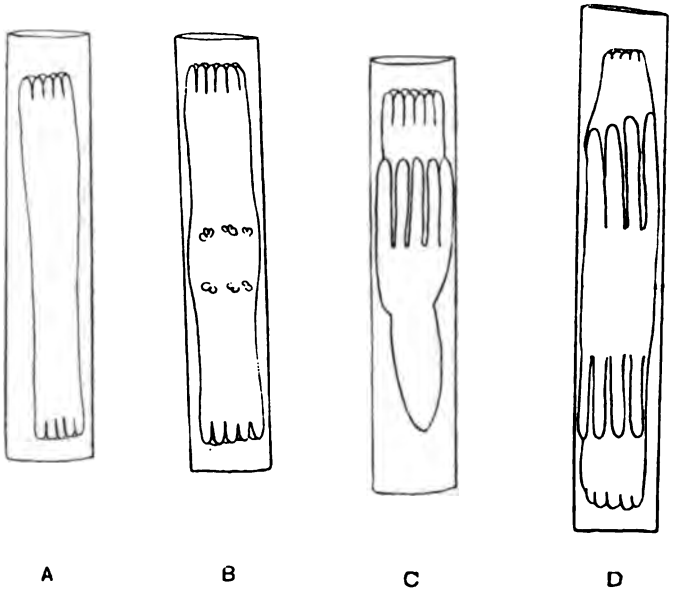
These tables give an unexpected verification of the conclusion doubtfully drawn from the first series. They seem to show that the length of the pieces from the distal region is of less importance than the kind of stalk from which the pieces are cut. Still I regard the results as too meagre to establish this view, however probable it may appear. Should more extensive experiments on material in better condition make certain this conclusion, they would make clearer the result, so often observed when short pieces are taken haphazard and all kept together, viz., that pieces of the same length often give rise to different structures, and pieces of very different lengths to similar structures, even when all the pieces have come numerically from similar distal regions.

A comparison between the lengths of the hydranth-forming region in pieces from the distal end of the stem and that of pieces from more proximal regions brings to light a somewhat remarkable result, viz., that although the length of the distal pieces may be three or four times as great as that which goes to form the hydranth in proximal regions, yet incomplete structures and incomplete double structures often develop. This is strikingly shown by comparing the hydranth forming area, measuring 20 divisions, of Fig. *L* (from a proximal part of the stem) with the lengths of some of the pieces in the first fifteen tables. In the latter there are cases in which only a hydranth forms in pieces measuring 95, 98 and even 105 divisions, which are four or five times as long as the length of the area in Fig. *L*.

There are also a number of cases in these tables in which proboscides develop in pieces measuring 40 and more divisions. In other words incomplete structures are produced by distal pieces that are several times longer than the average basal hydranth region. What is the meaning of this difference? One of the factors at least we can put our finger on. The thickness of the wall (cœnosarc) in the more distal region of newly formed stems is very much less than that in the basal region, so that quantitatively the lengths of pieces from these two regions do not represent the volume of the material contained in them. A longer distal piece may contain less material than a shorter basal piece. I do not doubt that other factors also come into play.

A similar phenomenon is found in regard to double structures formed in distal pieces. In Figs. *A*, *B*, *C*, *D* are shown four consecutive pieces of the same stem, measuring respectively 60, 62, 66, 75 divisions. In length these pieces are three times that of the hydranth-forming area of Fig. *L*. A half of any one of them would be longer than the entire hydranth region of the latter figure, yet the first and second of these pieces produced only double proboscides. Here also the only clue we have as yet to the result is the relative thinness of the cœnosarc in the more distal pieces.

Figs. *A* – *D*.



The Development of Short Pieces Closed at One End.

The development of double structures in many of the short pieces seems to be due to the pieces being open at both ends rather than to the small amount of material in the pieces. In order to make certain the truth of this conclusion I tested it by closing one end of short pieces. This is easily done by tying a silk thread tightly around a stem so that the cœnosarc of one side is separated from that of the other side, and then cutting off the stem of one side near to the knot. In experiments of this sort I found that incomplete structures develop if the piece is small enough, and double structures do not appear. The distal end of the incomplete structure is always turned towards the open end. The same result followed whether the short piece is on the distal or on the proximal side of the knot; in both cases the distal end of the complete and also of the incomplete structure is turned toward the open end of the piece. This shows that the influence that determines the distal end of the new structure comes from the open end of the piece, and that double structures do not develop when one end of the piece is closed.

Regeneration from the Attached Basal End of Branches.

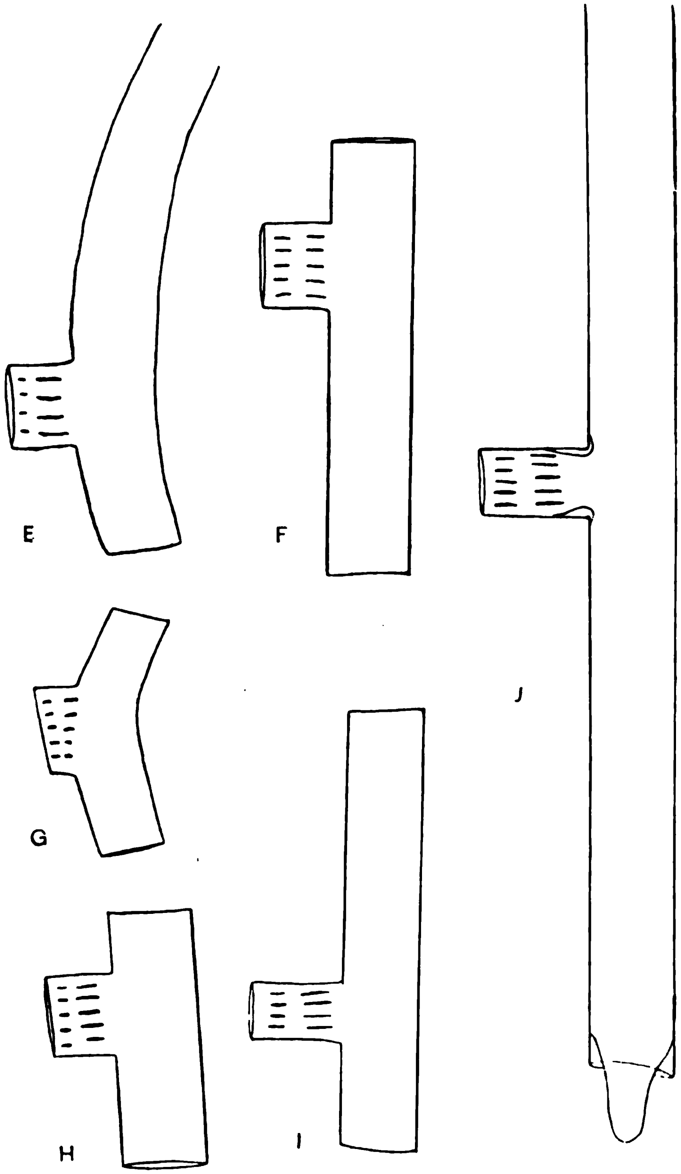
The presence of numerous side branches, even near the terminal hydranths, in *T. crocea* gives an opportunity of extending these experiments on Tubularia in another direction. If, for instance, a branch is cut off so near its origin that the short piece that remains is less than the average length of the hydranth-forming region (Fig. *E*), will an incomplete structure be produced, as happens when pieces of the same length are separated from the rest of the stem, or will the result be different owing to the connection of the short, lateral piece with the main stem? It would not be possible to state a priori how these short pieces attached at the side of the stem would behave, nor would our knowledge of the behaviour of grafted pieces help us in anticipating the result, since the orientation of lateral pieces is different from that of grafted ones.

The six figures represented in Figs. *E—J* show how short, lateral, attached pieces develop. It will be seen that although the pieces were sometimes only half or even less (only one third as long in Fig. *G*) as long as the typical hydranth-forming region[1]), yet they produced whole hydranths with both circles of tentacles; and in none of the cases observed was an incomplete structure, a proboscis for instance, produced. In a few cases I could see at first only a single circle of tentacles, but this proved to be the proximal circle, and the distal tentacles budded out at the cut-end. It appears that the connection of the base of the short piece with the main stem necessitates the formation of the proximal part of the hydranth. The number of cases

---

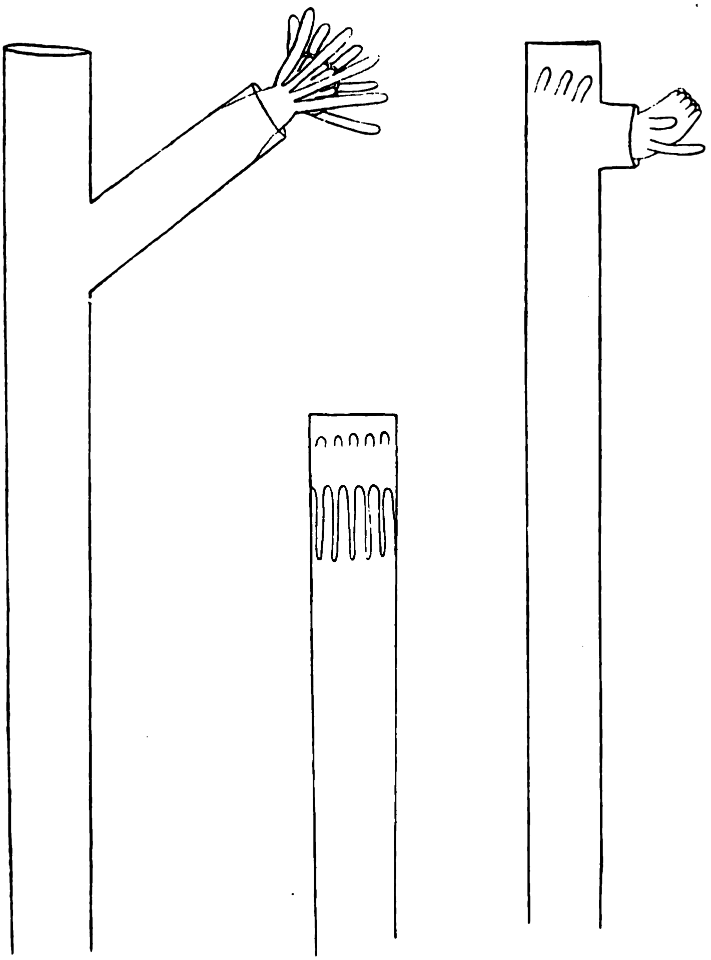
[1]) Compare with Fig. *L*.

Figs. *E*—*J*.



is, however, too small for me to state positively that incomplete structures never appear.

Figs. *K—M.*



In several other experiments the main stem was cut off near the origin of the branch as shown in Fig. *K.* In the first series the hydranth was left on the branch, and in none of the cases observed did a new hydranth develop on the end of the main stem[1]. In a parallel experiment both the main stem and the branch were cut off close to the origin of the latter, as shown in Fig. *M.* In some cases a new hydranth developed in the end-piece of the main stem, in others in the short piece of the branch, and in still others in both. When a hydranth developed in the end-piece of the main stem it did not extend behind the level of the branch, and when a hydranth developed in the branch it did not extend into the main stem, but when hydranths developed simultaneously in both main stem and branch, the proximal circle (or circles?) of tentacles sometimes appeared on the main stem in the region common to the two short ends, and often extended further downwards into the main stem. The same result is better shown in the cases to be next described in which short pieces were cut off near a branch.

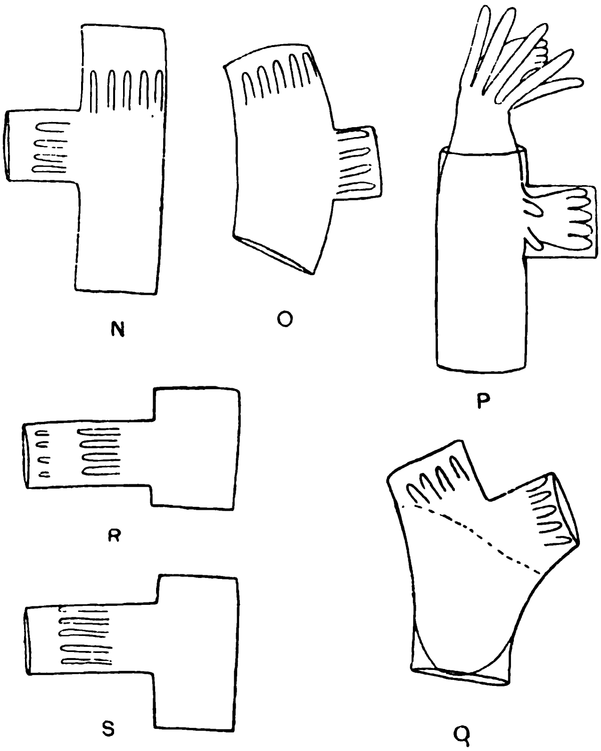
---

[1] The hydranths used were not in very good condition.

Short Pieces Cut Off Near a Branch.

Short pieces including the base of a branch and the part of the main stem that lies on each side of it were cut off. Some of the results are shown in Figs. *N* to *Q*. In Fig. *N* two hydranths are in process of forming. This is also the case in Fig. *O* in which the distal end of the stem and of the branch are very short. In these cases it seems probable that two whole hydranths would have formed each with it basal circle of tentacles (shown in the figures). In Figs. *P* and *Q* two hydranths are also forming. It should be noticed that in these latter two cases that the two ends in which

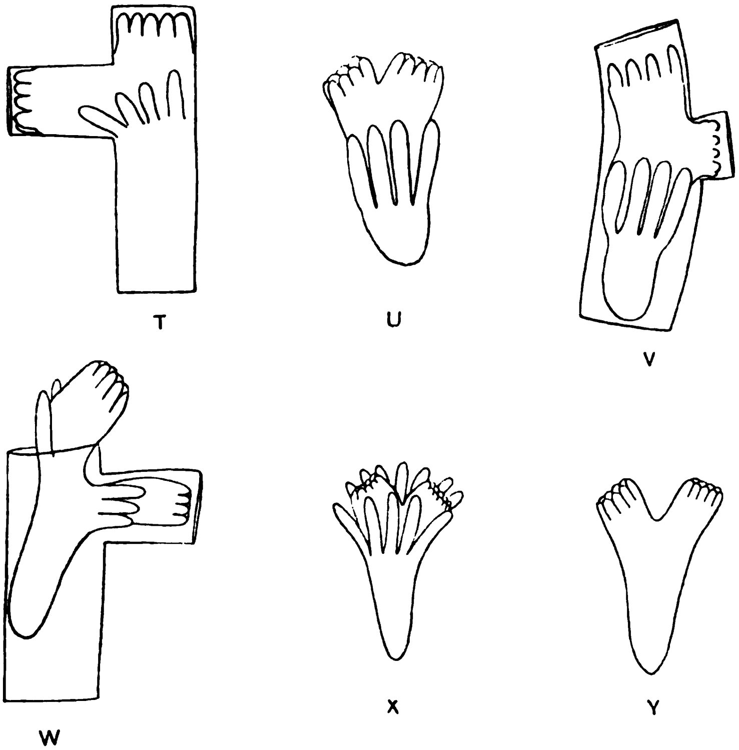
Figs. *N*—*S*.



hydranths are forming are of equal length. In Figs. *R* and *S*, on the contrary, the basal end of the branch was longer than the end of the stem, and the hydranth developed only in the longer piece.

In Fig. *T* (Figs. *T*—*Y* are from another series) two proboscides are present, one in the end of the stem and one in the branch. The proximal tentacles appear on the main stem in the region common to the two proboscides. When the latter hydranth was taken out of the perisarc it was found to have two proboscides and a common circle of tentacles around their base, Fig. *U*. A similar case is shown in Fig. *V*. In Fig. *W* two more or less complete hydranths are present. In the next case the proximal tentacles, belonging to both proboscides, were as in Fig. *V*. When removed from the tube the hydranth appeared as shown in Fig. *X*. Lastly in Fig. *Y* two proboscides developed, and there were no proximal tentacles present. The piece was quite a short one.

Figs. *T*—*Y*.



These experiments with short pieces including a part of a branch show one result that was not seen when a short piece was at the middle of a long stem, viz., that when two distal ends are of nearly the same length each may produce only the distal part of a hydranth. The proximal part of the hydranth is formed either in the region common to the two proboscides, or even in the more proximal part of the stem. Should a more extensive series of experiments show that the differences that have been found between the behaviour of a short piece of a branch lying in the middle of a stem, Fig. *J*, and one lying near the cut-end of a stem, Figs. *M* and *T*, are constant it will then be time for further speculation as to the meaning of this difference, but until more experiments have been made it is safer, I think, to refrain from theorizing in regard to the phenomenon.

The Method of Closing of a Cut-End.

When the stem is cut in two there are exposed at the cut-end the perisarc, the cœnosarc composed of ectoderm and endoderm, the ends of the two (or four) longitudinal ridges formed by folds of endoderm, and the central cavity filled with fluid. In the course of a few minutes the cœnosarc withdraws slightly from the cut-end of the perisarc and then a thin membrane develops that ultimately closes the end. This closing has been already mentioned by Bickford, Driesch, and myself, but the process has been insufficiently described. A closer examination has shown that a very remarkable and unique change takes place, for the rough cut-end does not bend or arch over the opening of the tube, but a thin membrane develops near the end, which extends squarely across the end. This membrane is composed of ectoderm on its outer and endoderm on its inner surface. The central rim of the membrane is smooth and continuous. It does not have a ragged edge like that left at the cut-end of the cœnosarc. In fact sections show that the margin of the membrane is largely a new formation and not simply the cut-end of the cœnosarc. The membrane develops just a little behind the cut-end, so that for a time at least some of the ectodermal cells continue outwards beyond the level of the membrane. These seem to be in part cells left behind as the new edge develops. In the course of twenty or thirty minutes the membrane closes in the center as a result of the advance of its margin. All trace of the opening is soon lost, but for a time radiating lines, indicating cell-boundaries, extend from a central point to the perisarc. By killing pieces in process of closing, with a weak solution of osmic acid, rapidly washing in distilled water and staining for a few minutes (in the sunlight) in a one per cent solution of silver nitrate, the boundaries of the superficial (ectodermal) cells can be distinctly seen[1]. The ectoderm is found to be composed of a large number of cells, somewhat elongated in a radial direction. There are a number of rows of cells between the center and the outer border, i. e. individual cells do not extend from center to periphery.

The rapidity of closing, and the absence of dying material along the edge at once attract attention, and in the absence of definite muscle-fibres in the stem, the method of closing presents some interesting problems. By means of a few experiments I tried to determine the nature of the closing process. It seemed a priori possible that loss of water, or even absorption of water at the cut-surface might be a factor in the result. If this were so the rate of closing should vary with the density of the sea water. Some pieces were placed in sea water diluted one-third with fresh water, others in sea water to which one per cent sodium chloride had been added, and still others in normal sea water. No difference in the rate of closing of the pieces could be detected. By the addition of eight per cent of cane sugar to sea water the osmotic pressure is materially altered, yet the closing in of pieces placed in this solution was about the same as in sea water.

It has been stated that the addition of one per cent sodium chloride to the sea water did not affect the closing of the pieces. Even one and a half per cent did not prevent the closing in all cases, although many of the pieces were injured, and if two per cent were added the pieces died. LOEB has shown that sodium chloride has a specific action on muscular contractility, but not on amoeboid movements. The behaviour of the pieces in the presence of this salt suggested that the closing-process is some sort of amoeboid movement, involving the cœnosarc as a whole rather than individual cells. My observations of the normal closing had suggested the same view to me before I tried the action of the solutions. I think it very probable that the closing-process involves an amoeboid-like extension of the cœnosarc across the open end. If this is the correct interpretation we find here a new kind of contraction phenomenon, and one deserving of a much more extended examination, especially as there is reason to believe that the process is of wide occurrence in lower animals and perhaps in higher ones also, especially in embryonic stages. I have also studied the closing of the surface left after cutting off a large piece of the side of young tadpole-embryos, where a process, that appears to be similar to that in Tubularia, takes place. The large wounds thus formed close rapidly without the formation of necrotic tissue at the cut-edge — the ectoderm advancing with an even, clean margin towards the center. In a future paper I shall hope to describe the process in the embryo of the frog more fully.

---

[1] Especially in pieces cut off obliquely in which the closed end can be more easily examined.

Summary.

1) Consecutive, short pieces of the same stem of Tubularia cut from the region behind the hydranth show a tendency to produce similar structures. The different behaviour of different stems is probably due to differences in the age of the stem. As a stem gets older its cœnosarc becomes thicker.

2) Pieces from the distal end of the stem, four or five times longer than the average hydranth-forming region of the more proximal region, often produce only incomplete structures. The explanation of this may depend, in part, on the thinner cœnosarc of the more distal (newer) region. Quantitatively a longer piece from the distal region corresponds to a shorter piece of the proximal region.

3) When double structures are produced the same structure always appears at both ends. This holds for all kinds of incomplete structures, as well as for whole ones. The explanation may be found in the action of the same influences on the two free ends, and also in the interaction of the developing material of the two ends on each other.

4) When a branch is cut off close to the main stem, as shown in Figs. *E—J*, there develops from the short, attached basal piece of the branch a whole hydranth, and not an incomplete structure, even when in size the piece is much less than the average hydranth-forming region. The connection of the short piece at its base with the main stem appears to influence the developing region in such a way that the proximal end of the hydranth is formed, hence incomplete, distal structures are not produced, as when the piece is isolated.

The basal tentacles appear at the base of the piece of the branch and not in the main stem.

5) Short pieces closed at one end produce single (i. e. not double) structures that are often incomplete. The result shows that the distal end of the new structure is determined by external influences.

6) When both the main stem and a branch are cut off near the origin of the latter (Fig. *T*), each end may produce a whole hydranth or a proboscis. If the latter, the proximal tentacles may appear on the main stem in the region between the two hydranths or even proximally to the origin of the branch.

7) The closing of the cut-end of a piece by means of a double plate (composed of ectoderm and endoderm) is not due to the bending over of the cut-edge, or to the action of constricting muscles or fibres, but to a peculiar sort of contractile movement characterized by the regulated advance of the whole material and not of the cells as individuals, also by the formation of a smooth edge which moves towards the center where the final closure takes place. During the closing some of the cells are carried from the periphery out upon the surface of the closing membrane.

Zusammenfassung.

1) Wenn auf einander folgende, kurze Stücke desselben Stammes von Tubularia aus der Gegend hinter dem Hydrant geschnitten werden, so zeigen sie die Tendenz, ähnliche Strukturen hervorzubringen. Das verschiedene Verhalten verschiedener Stämme beruht wahrscheinlich in Altersverschiedenheiten. Mit zunehmendem Alter eines Stammes wird sein Cönosark dicker.

2) Distal gelegene Stammstücke, vier bis fünfmal länger als der durchschnittlich den Hydrant bildende Bezirk, bringen es oft nur zu unvollständigen Neubildungen. Eine Erklärung dafür könnte theilweise in der dünneren Beschaffenheit des Cönosarks nach dem distalen (neueren) Ende hin liegen. Quantitativ entspricht ein längeres distal gelegenes Stück einem kürzeren aus einem proximalen Bezirk.

3) Findet Neubildung an beiden Schnittflächen statt, so erscheint stets dieselbe Struktur an beiden Enden. Man könnte dafür eine Erklärung in dem Einfluss gleicher Bedingungen für beide freie Enden und auch in einer gegenseitigen Beeinflussung des Materials an beiden Enden erblicken.

4) Schneidet man einen Zweig kurz am Hauptstamme ab (vgl. Fig. *E—J*), so entwickelt sich aus dem kurzen, angewachsenen Fußende des Zweiges ein ganzer Hydrant, keine unvollständige Neubildung, auch wenn das Stück viel kleiner ist als der gewöhnlich den Hydrant bildende Bezirk. Die basale Verbindung des kurzen Stückes mit dem Hauptstamm scheint den Bildungsbezirk in der Weise zu beeinflussen, dass das proximale Ende des Hydrants gebildet wird und weiterhin keine unvollständige distale Neubildung mehr entsteht, wie

sonst wenn es sich um ein abgelöstes Stück handelt. Die basalen Tentakel erscheinen am Grunde des Zweigstückes und nicht im Bereich des Hauptstammes.

5) Kurze, an einer Seite geschlossene Stücke erzeugen einfache (d. h. nur an einer Stelle) Neubildungen, die häufig unvollständig sind. Dieser Ausgang zeigt, dass das distale Ende der Neubildung von äußeren Einflüssen regiert wird.

6) Wenn sowohl der Hauptstamm als ein Zweig nahe am Ursprung des letzteren abgeschnitten wurden (Fig. *T*), so kann jeder Stumpf einen ganzen Hydrant oder auch nur einen Rüssel hervorbringen. Ist das letztere der Fall, so können die proximalen Tentakel am Hauptstamm zwischen den beiden Hydranten oder sogar nahe am Zweigursprunge zuerst auftreten.

7) Der Schluss einer Schnittfläche eines Stückes mittels einer doppelten (aus Ekto- und Entoderm gebildeten) Zellplatte kommt nicht durch Zusammenbiegen der Schnittränder zu Stande, auch nicht durch die Wirkung zusammenziehender Muskeln oder Fibrillen, sondern durch eine eigenthümliche Art von Kontraktionsbewegung, welches sich durch bestimmt vorgezeichnete Bewegung des ganzen Materials und nicht der Zellindividuen charakterisirt, und durch die Bildung eines glatten Wundrandes, welcher beim definitiven Wundschluss centralwärts zusammenrückt. Während des Schlusses trennen sich viele Zellen von der Peripherie ab, indem sie auf die Oberfläche der Verschlussmembran gelangen.

Experimental Studies on the Formation of the Embryo of Bufo lentiginosus.

By

Helen Dean King.

With 45 figures in text.

Eingegangen am 17. December 1901.

Much of the experimental work on the gastrulation of the amphibian egg has been done by puncturing the egg with a fine needle or hair, thus forming an extra-ovate which serves as a fixed point for orientation during the formation and closure of the blastopore. This method has been criticised by SCHULTZE (8) and others because of its supposed interference with normal development, and also because of the frequent lack of uniformity in the results. Against the first criticism can be urged the fact that embryos, apparently perfectly normal in every respect, develop from eggs which have been operated upon in such a way; while slight differences in the location of the puncture in the various eggs of a series will account for much of the diversity in results.

The experiments recorded in the present paper were made in the Biological Laboratory of Bryn Mawr College during the spring of 1901. I am indebted to Prof. T. H. MORGAN for many helpful suggestions during the progress of my investigations.

Method.

In making the following experiments a greater part of the jelly-like substance surrounding the egg was first removed and the egg was then put in a shallow dish with a layer of paraffine in the bottom. The dish was subsequently placed on the stage of a dissecting microscope and the egg, barely covered with water, was held by fine forceps and punctured with a sharp needle.

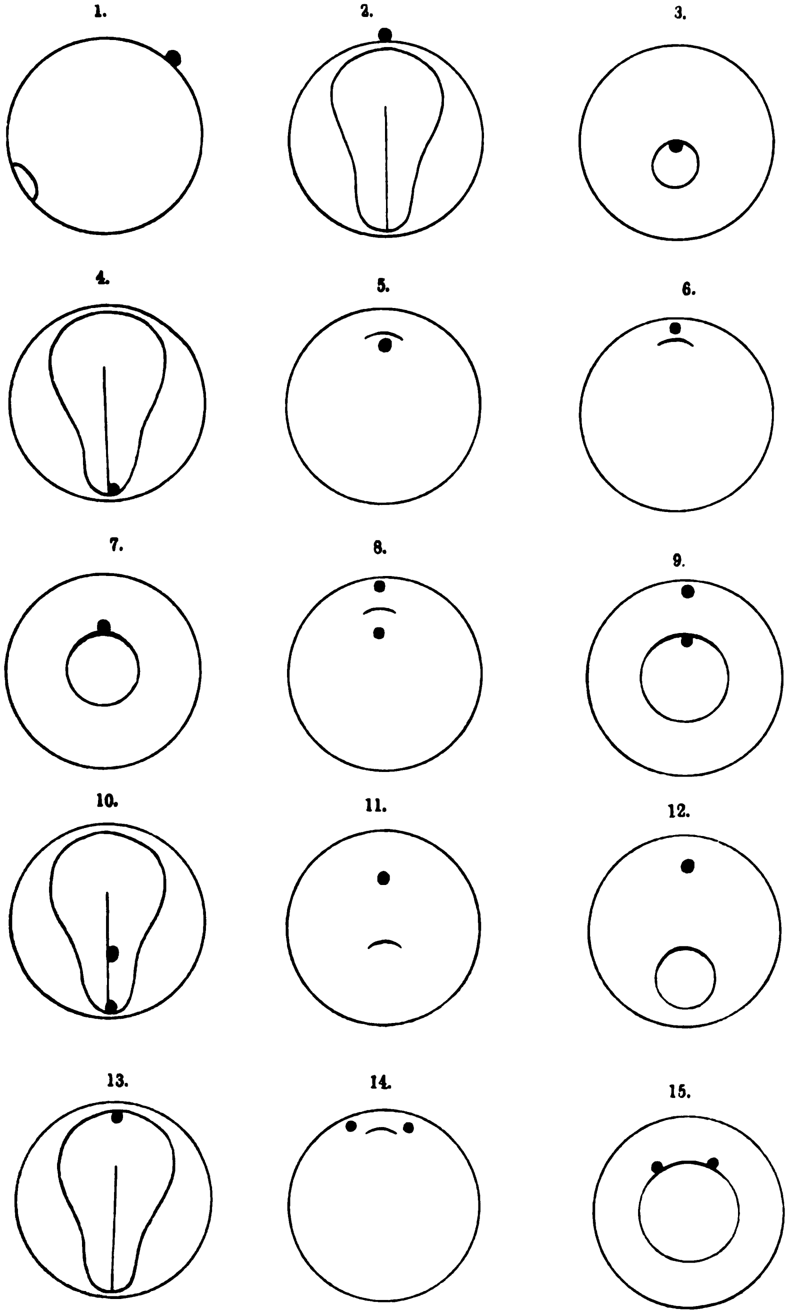
An extrusion of egg substance always follows the puncture. If the extra-ovate thus formed is very small, the normal development of the egg does not seem to be interfered with and the extra-ovate remains attached to the egg in a great majority of cases. If an extensive injury is made so that a large extra-ovate is formed, spina bifida embryos are produced in some cases, and in others the extra-ovate separates from the egg in the course of a few hours and later both the extra-ovate and the egg disintegrate. Normal development is so rare in cases where the extrusion of a considerable amount of egg substance follows the operation that in all the following experiments the eggs were discarded if a very large extra-ovate formed. To this is probably due the uniform results that were obtained in a majority of cases. Where, in different sets of experiments, the results are given for only a portion of the eggs operated on, it is to be understood, unless otherwise stated, that in the remaining cases the extra-ovate became detached and so no definite conclusions were possible in regard to these eggs. After the operation the eggs were transferred into dishes filled with fresh spring water and observations were made at frequent intervals during the next twenty-four hours.

a. Sticking the Egg Before the Appearance of the Blastopore.

Experiment 1. Twenty-five eggs were punctured as nearly as possible in the centre of the black hemisphere just before the appearance of the blastopore. Eight hours later the blastopore was closing in and the extra-ovate in every case was about 180° from it (Fig. 1). The slight variations noted in the position of the extra-ovates were undoubtedly due to the fact that the puncture had not always been made exactly in the centre of the black hemisphere. The morning following the operation the extra-ovate in 18 cases appeared very nearly as shown in Fig. 2, i. e. it was a little in front of the transverse portion of the neural folds at the anterior end of the embryo. In the remaining embryos, the extra-ovate was farther away from the neural folds, being in 3 cases in the region of the suckers. The results of this experiment show that the anterior end of the embryo in the Bufo egg does not extend up to the centre of the black hemisphere but lies somewhere below it.

Experiment 2. In 24 eggs an extra-ovate was produced as

Diagram I.



nearly as possible in the centre of the white hemisphere just before the dorsal lip of the blastopore formed. Eight hours after the operation the eggs appeared as in Fig. 3. The blastopore was closing in and the extra-ovate was at the edge of the dorsal lip. When the neural folds formed, the extra-ovates in the 19 eggs which were developing normally were at the posterior end of the embryo (Fig. 4). This experiment shows that the dorsal lip of the blastopore moves backward at least to the centre of the white hemisphere and if it goes beyond this point that it carries the ovate with it.

b. Extra-ovates Produced in Various Parts of the Egg When the Dorsal Lip of the Blastopore is Just Appearing.

Experiment 3. At 9 a. m. on April 23rd, 20 eggs were stuck just below the middle of the dorsal lip of the blastopore as the latter was first appearing (Fig. 5). Two hours later the extra-ovate in every instance was found to be at the edge of the blastopore. In this set of experiments, if the extra-ovate was even moderately large, abnormal development almost invariably resulted. If, however, the extra-ovate was very small as was the case in 14 of the eggs operated on, development apparently proceeded in a normal manner and the extra-ovate remained attached to the egg. When the blastopore was closing in, the extra-ovate was found at the edge of the dorsal lip as in Fig. 3, and it subsequently occupied a position at the posterior end of the neural folds (Fig. 4). This experiment shows that the dorsal lip of the blastopore moves over the lower hemisphere of the egg and that it carries the extra-ovate with it after the latter has been reached by the advancing lip.

Experiment 4. In 20 cases the eggs were punctured just above the middle of the forming blastopore (Fig. 6). Six hours later the extra-ovate occupied a position at the edge of the dorsal lip as shown in Fig. 7. The next morning, in the 16 eggs in which the extra-ovate still remained attached to the egg, its position was invariably at the posterior end of the neural folds as in Fig. 4. It is evident from this result that the extra-ovate was produced in a region of the egg where the cells move in towards the blastopore rim and that, as the extra-ovate was too large to turn under with the in-rolling cells, it kept its position at the edge of the blastopore during the subsequent changes in the egg.

Experiment 5. The operations in experiments 3 and 4 were combined in another experiment on a set of 24 eggs. Two punctures were made, one a short distance below the middle of the dorsal lip of the blastopore, the other an equal distance above the middle of the dorsal lip (Fig. 8). The extra-ovates were however, not quite as close to the blastopore as in the two preceding experiments. Six hours after the operation, when the blastopore was circular and beginning to close in, the extra-ovates in 18 eggs occupied a position as seen in Fig. 9. The extra-ovate which had been made below the blastopore had been reached by the advancing dorsal lip and it occupied a position on the dorsal rim of the blastopore. The other extra-ovate was evidently made outside of the region where the cells move in towards the edge of the blastopore as it had remained stationary and was much farther away from the dorsal lip than when the operation was first made. Twenty-four hours after the operation, the extra-ovates were still attached to the eggs in 12 cases and their position was that seen in Fig. 10. One extra-ovate was, as expected, at the caudal end of the embryo. The position of the other extra-ovate varied slightly in different eggs being sometimes nearer sometimes farther away from the posterior end of the neural folds than the extra-ovate shown in Fig. 10, but in all cases it occupied a position somewhere near the middle region of the embryo. The variations in the position of this extra-ovate can be attributed, I think, to slight variations in the positions of the punctures when the operations were made. It is extremely difficult, as no doubt other investigators have found, to puncture a given series of eggs in exactly the same place each time, and very slight variations in the position of the extra-ovate at the beginning of an experiment might produce a marked difference in the location of the extra-ovate several hours later.

The inference that can be deduced from the foregoing experiment is that the region of the egg a little above the middle of the dorsal lip of the blastopore becomes the middle portion of the embryo and that the cells in this region do not move backward to the rim of the blastopore.

Experiment 6. In 8 cases the eggs were stuck as in the preceding experiment but the extra-ovates were intentionally produced as close to the edge of the blastopore as they were in experiments 3 and 4. In every instance the extra-ovates were found at the edge of the dorsal lip in the course of a few hours, and the next morning they were both at the posterior end of the neural folds as in Fig. 23. This result simply confirms those obtained in Experiments 3 and 4 and shows that the formation of two small extra-ovates in the egg does not interfere with development any more than does a single puncture.

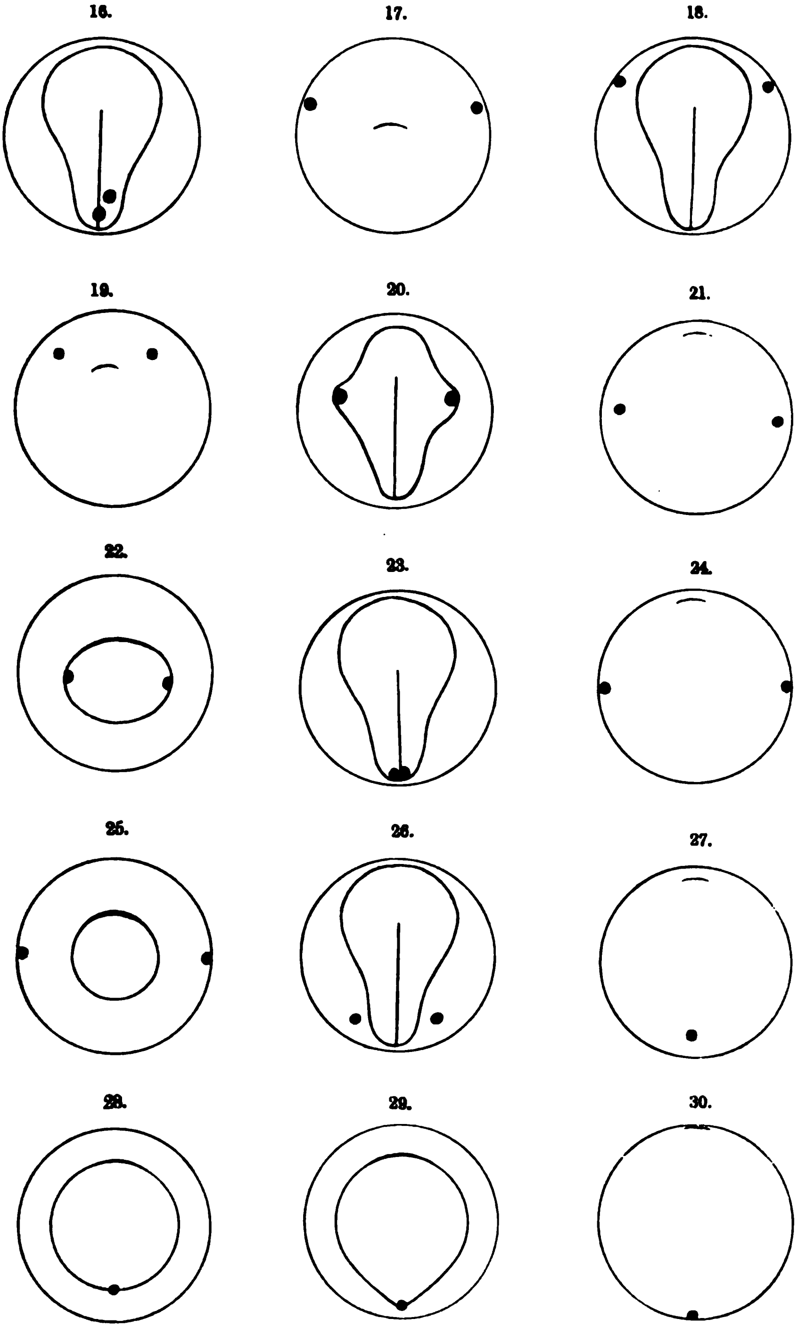
Experiment 7. The object of this experiment was to locate, if possible, the region from which the cephalic portion of the embryo is formed. Experiment 1 showed that the anterior end of the embryo does not extend up to the centre of the black hemisphere, and Experiment 5 indicates that the middle part of the embryo comes from the region a little above the dorsal lip of the blastopore. In the following experiment 26 eggs were stuck as nearly as possible half way between the pole of the black hemisphere and the dorsal lip of the blastopore (Fig. 11). When the blastopore was closing in, the extra-ovate in every case, was farther from the dorsal lip than in the beginning of the experiment, again showing that the blastopore lip moves backward over the yolk (Fig. 12). When the neural folds appeared some hours later, the extra-ovate in 19 eggs was at or very close to the transverse portion of the neural folds at the anterior end of the embryo (Fig. 13).

The conclusion from this experiment is, obviously, that the anterior end of the embryo in the egg of Bufo is formed from the region about half way between the centre of the black hemisphere and the place below the equator of the egg where the dorsal lip of the blastopore first appears. The embryo is thus formed partly at least, on the black hemisphere as maintained by MORGAN, EYCLESHYMER and WILSON.

Experiment 8. After the appearance of the dorsal lip of the blastopore, 15 eggs were punctured in the black hemisphere as nearly as possible 180° from the edge of the blastopore. The result in every case was very similar to that obtained in Experiment 1; the extra-ovate was found in the vicinity of the suckers, never at or very close to the anterior end of the neural folds.

Experiment 9. In Experiments 4 and 6 the cells just above the dorsal lip of the blastopore were found to be inside the region of cell activity and to move backward to the edge of the blastopore. In order to ascertain whether cells from the sides also move in towards the rim of the blastopore 22 eggs were stuck on either side of the blastopore lip as seen in Fig. 14. The extra-ovates were made quite close to the edge of the blastopore, but in every case they were formed of the deeply pigmented cells above the line of the dorsal lip. Six hours later the extra-ovates were found on the

Diagram II.



edge of the blastopore in every one of the 18 eggs to which they were still attached (Fig. 15). The following day the extra-ovates occupied a position at or very near the posterior end of the embryo (Fig. 16). This experiment shows that the cells at the sides as well as those just above the dorsal lip move to the edge of the blastopore. The extra-ovates, being too large to turn under with the invaginated cells, keep their position at the edge of the dorsal lip and so are found later at the caudal end of the embryo.

Experiment 10. On April 26th, 18 eggs were operated on in the following manner: each egg was rotated until the white hemisphere appeared uppermost and then a puncture was made at the outer edge of the circumference of the egg on either side as seen in Fig. 17, the extra-ovates being formed in the black cells. The experiment was made to see if the cells from this region 90° away from the dorsal lip of the blastopore are brought in to the median line during the closure of the lips of the blastopore to help form the mid-dorsal portion of the embryo. When the ventral lip of the blastopore formed, extra-ovates were attached to the eggs in 16 cases and they had apparently remained stationary. On April 27th, the extra-ovates in every one of the 12 developing eggs were found to be outside of the neural plate (Fig. 18).

A slight variation of the above experiment was made on 10 other eggs. The eggs were rotated as before so that the dorsal lip of the blastopore came to occupy the highest point of the egg and then two punctures were made, one on either side of the blastopore, the extra-ovates being formed much nearer the edge of the blastopore than before (Fig. 19). The result was practically the same as in the previous experiment. The extra-ovates appeared to be stationary during the closing of the blastopore and later were found outside the neural plate but closer to it than were the extra-ovates in the previous case. When, in a third set of 8 eggs, the punctures were made still nearer to the lateral lips of the blastopore, the extra-ovates were later found inside the neural plate, but they interfered with the normal development of the neural folds as the latter were greatly distorted (Fig. 20), and abnormal embryos resulted in every case.

The results obtained in this group of experiments indicate that but a comparatively small amount of material from the dark hemisphere at the sides of the dorsal lip of the blastopore moves in during the closure of the blastopore to become part of the mid-dorsal region of the embryo. The cells in the region where the extra-ovates were formed in the two first experiments in this group remained practically stationary during the closure of the blastopore and were later found outside the neural plate at the sides of the embryo. The presence of the extra-ovates certainly does not prevent a movement of the cells as Experiments 4, 6, and 9 testify, and in this series of experiments perfectly normal embryos resulted in every case except in the last set of 8 eggs where the extra-ovates were evidently made so close to the region of moving cells that they were pulled into the neural plate causing abnormal development.

Experiment 11. In 14 cases the eggs were punctured at the extreme edge of the white area about 90° away from the blastopore as shown in Fig. 21. The experiment was made to ascertain how much of the white area in the normally pigmented eggs is included in the lateral extension of the lips of the blastopore. In every case the normal formation of the lateral lips of the blastopore was prevented as the blastopore, instead of appearing almost perfectly round as it usually does, was a decided oval after the ventral lip had formed being drawn out laterally by the presence of the extra-ovates (Fig. 22). In 8 cases normal embryos were formed later with the extra-ovates at the posterior end of the neural folds (Fig. 23). In the remaining cases in this set of experiments, the eggs died or spina-bifida embryos resulted.

The normal formation of the blastopore was interfered with by this experiment so that no definite conclusions can be drawn from the results. It seems probable, however, that the extra-ovates had been made in a region of cell activity and that their presence at first retarded the movement of the cells. In the eggs that later appeared to develop in a normal manner the extra-ovates were moved in to the median line (Fig. 23) indicating concrescence of material from the sides of the blastopore lips. Incidentally this experiment shows the migration of the dorsal lip of the blastopore over the lower hemisphere as a comparison of Figs. 21 and 22 will show that the dorsal lip has advanced much nearer the centre of the white hemisphere.

Experiment 12. In order not to interfere with the normal formation of the lateral lips of the blastopore as was done in the previous experiment, and to determine how much material moves in to the lateral lips during the time the blastopore is becoming circular, 18 eggs were stuck as in Fig. 24, the extra-ovates being produced at the outer circumference of the field and always within the black cells. Thirteen eggs gave satisfactory results. Seven hours after the operation the eggs appeared as in Fig. 25. The lateral and ventral lips of the blastopore had formed in the normal manner and the extra-ovates in every case were some distance from the blastopore rim. The day following, the neural folds had appeared and the extra-ovates were near the posterior end of the embryo but outside the neural folds (Fig. 26). It is evident from this experiment that the extra-ovates were not formed within the region of cell activity and therefore that material from this part of the egg is not brought to the median line during the closure of the blastopore.

Experiment 13. To determine the region of the egg involved in the formation of the ventral lip of the blastopore, 23 experiments were made in which the egg was punctured at the edge of the white area, directly opposite the middle of the dorsal lip of the blastopore, and about 160° away from it (Fig. 27). Four hours after the operation the ventral lip of the blastopore had formed, and in every case the extra-ovate occupied a position at the edge of the blastopore rim (Fig. 28), showing that the cells at the edge of the white area are unquestionably involved in the formation of the ventral lip of the blastopore.

Experiment 14. On April 25th, 18 eggs were punctured, as in the preceding experiment, opposite to the dorsal lip of the blastopore; but in this set the extra-ovates were produced just within the black cells and therefore about 170° from the blastopore. When the blastopore rim had extended to this region, the presence of the extra-ovate interfered with the normal formation of the ventral lip and the latter was drawn out of shape as seen in Fig. 29 making the lower part of the blastopore rim decidedly pointed. Later, however, the blastopore rim rounded up in 11 cases and further development appeared to be perfectly normal, the extra-ovate being located at the posterior end of the embryo when the neural folds were formed.

The normal formation of the ventral lip of the blastopore was interfered with in this experiment so that, although later development seemed perfectly normal, no definite conclusion can be drawn from the results any more than from the results of Experiment 11, yet one may, perhaps, be justified in inferring that the region involved in the formation of the ventral lip of the blastopore does not extend much beyond the edge of the white area.

Experiment 15. In 16 cases a puncture was made opposite the dorsal lip of the blastopore and quite 180° away from it (Fig. 30). Subsequently the extra-ovate appeared to be stationary and in the 11 eggs which developed, the extra-ovates were always found some little distance from the ventral lip. The results of this experiment when combined with those of Experiments 13 and 14 lead to the conclusion that only the cells at the edge of the white area take part in the formation of the ventral lip of the blastopore and that the region involved in this process does not extend very far within the black cells.

c. Sticking the Egg After the Formation of the Ventral Lip of the Blastopore.

These experiments were made to determine, if possible, the relative rate of movement of the dorsal and of the ventral lip of the blastopore and the manner of closure of the blastopore, as well as to confirm in certain cases the results obtained from the previous experiments.

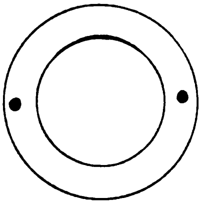
Experiment 16. Soon after the formation of the ventral lip, 12 eggs were punctured as in Fig. 31, the extra-ovates being produced at equal distances from the lateral lips of the blastopore and very near to the outer circumference of the egg. In every case the extra-ovates appeared to be stationary, the blastopore lips closing in as in Fig. 32. The results of this set of experiments lead to the same conclusion as that drawn from Experiment 12, namely that the region of the egg where the extra-ovates were formed is not involved in the formation of the blastopore ring.

Experiment 17. In another set of 10 eggs, extra-ovates were produced as in the previous experiment, but they were quite close to the lateral lips of the blastopore. Three hours later the blastopore had decreased considerably in size and in every case the extra-ovates had the same position with respect to the new blastopore rim that they had when the operation was made (Fig. 33), showing that they had been formed in a region of cell activity and had been moved in with cells which are brought to the median line by the closure of the blastopore to help form the embryo by a process of concrescence as maintained by Roux (7) and Morgan (5, 6).

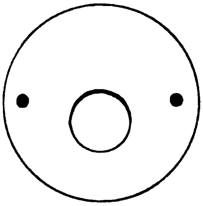
Experiment 18. If a puncture is made a short distance above the dorsal lip of the blastopore and another at an equal distance below the ventral lip (Fig. 34), the result depends on the distance of the punctures from the rim of the blastopore. If the extra-ovates

Diagram III.

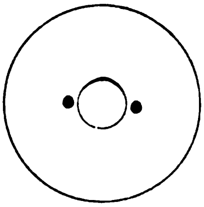
31.



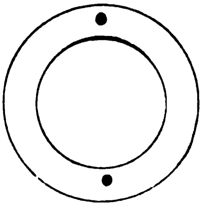
32.



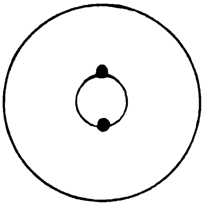
33.



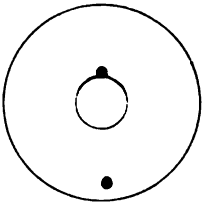
34.



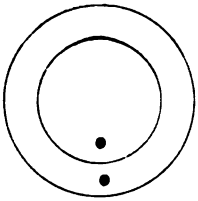
35.



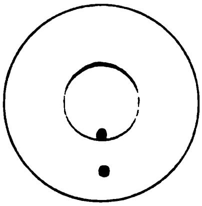
36.



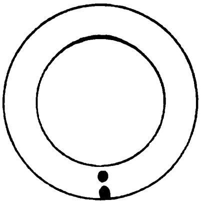
37.



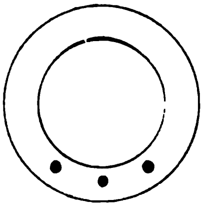
38.



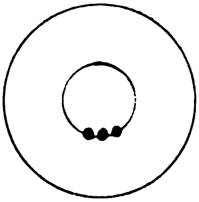
39.



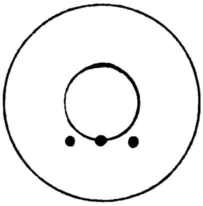
40.



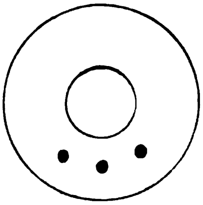
41.



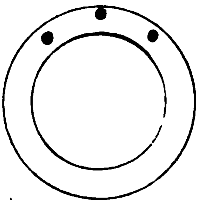
42.



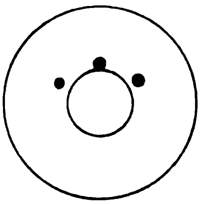
43.



44.



45.



are quite close to the edge of the blastopore they later come to occupy a position at the blastopore rim (Fig. 35). This result was obtained in 9 out of 13 experiments. When, in an equal number of cases, the extra-ovates were produced somewhat farther away from the rim of the blastopore, the result in the 7 cases where the extra-ovates remained attached to the egg was that seen in Fig. 36. The upper extra-ovate was found at the edge of the dorsal lip of the blastopore, while the lower extra-ovate had apparently remained stationary and was much farther away from the ventral lip than when the operation was first made. This experiment shows that the ventral as well as the dorsal lip of the blastopore moves over the yolk, but that a greater amount of material is involved in the dorsal than in the ventral movement. The forward movement of the ventral lip of the blastopore is still further indicated by the following experiment.

Experiment 19. Eight eggs were punctured as shown in Fig. 37. The extra-ovates being at equal distances from the edge of the ventral lip, one being within the yolk plug, the other below the ventral lip in the black cells. Six eggs developed as in Fig. 38. The ventral lip advanced sufficiently to meet the inner extra-ovate, while the other extra-ovate being beyond the region of moving cells, remained stationary. The apparent movement of the ventral lip is not due to a sliding backward of the yolk cells bringing the inner extra-ovate in contact with the stationary ventral lip. This is shown by the fact that the distance between the lower extra-ovate and the edge of the ventral lip does not remain constant during the closure of the blastopore. There is a decided increase in the space between the lower extra-ovate and the ventral lip during this time which can only mean that the ventral lip has moved forward over the yolk since it was first formed at the edge of the white area.

Experiment 20. In a set of 10 eggs, two very small punctures were made, both below the ventral lip of the blastopore, one extra-ovate was quite near the edge of the blastopore, the other was at the outer circumference of the egg when the blastopore occupied the highest point of the egg (Fig. 39). In 7 of the eggs the extra-ovate nearer the ventral lip disappeared, presumably it had been turned under the rim of the blastopore with the invagination cells. The other extra-ovate remained stationary and in later stages it was farther away from the blastopore rim than when the operation was made. In the other three eggs in this set, the extra-ovates were somewhat large and three hours after the operation the egg appeared as in Fig. 38. The inner extra-ovate was too large to be turned under with the in-rolling cells and so remained at the edge of the blastopore, while the other ovate appeared as in the remaining eggs of the set.

Experiment 21. Thirty eggs were punctured as in Fig. 40, three small extra-ovates being formed as nearly as possible at equal distances from the ventral lip. Satisfactory results were obtained in only 9 cases, the other eggs dying or development becoming abnormal. In 4 of the 9 cases, all of the extra-ovates were later found at the edge of the ventral lip (Fig. 41), indicating that the entire region where the ovates were formed had moved forward at an equal rate to the ventral lip. In 2 other eggs the middle extra-ovate was found at the edge of the blastopore, the other two being some distance away (Fig. 42). In the remaining 3 cases the punctures had been made farther away from the edge of the blastopore than in the preceding eggs and had presumably been made outside the region of cell activity as the extra-ovates remained stationary during the forward movement of the ventral lip keeping their same relative position with one another (Fig. 43).

The results of this experiment are, unfortunately, open to two different interpretations. The conclusion from four of the eggs is, apparently, that the material at the sides and below the ventral lip moves forward at an equal rate to the edge of the blastopore, while the results in 2 cases seem to show that the material just below the middle of the ventral lip moves faster than that at the sides. Slight variations in the position of the punctures when the operation was made would account for the difference in the result in these two cases, and it seems probable that the difference in the rate of movement, if it does exist, is very small and of comparatively little importance.

Experiment 22. In order to determine, if possible, whether the material just above the dorsal lip of the blastopore moves more rapidly than that at the sides, 15 eggs were punctured as shown in Fig. 44, three extra-ovates being produced at equal distances from the dorsal lip when the ventral lip had just formed. The results from this experiment were most satisfactory as in every one of the 8 cases in which the ovates remained attached to the egg the position of the ovates was that seen in Fig. 45. The middle extra-ovate was always at the edge of the dorsal lip, the other two extra-ovates were about as far from the blastopore rim as they were when the operation was first made, thus showing that they had been carried along with the moving cells in this region. The experiment shows without question that the material above the middle of the dorsal lip moves more rapidly than that at the sides. It is also evident that a more extensive region is involved in the movement at the dorsal lip of the blastopore than at the ventral lip, as the extra-ovates were formed about the same distance from the dorsal lip in the present experiment as they were from the ventral lip in the last experiment where they remained stationary during the closure of the blastopore.

Experiment 23. When the ventral lip had just formed, a puncture was made as nearly as possible in the centre of the yolk plug in 20 eggs. In the 18 eggs that continued developing the ovate was in every case in, or very close to, the centre of the yolk plug during all stages of the closure of the blastopore thus showing that the blastopore lips close in at nearly equal rates from all sides.

Experiments similar to most of the foregoing have been made by other investigators and have led to varying results. ROUX was the first to carry out experiments of this nature. He found that an injury to the dorsal lip of the blastopore in the egg of Rana produced a defect in the brain region of the embryo, and from this result he inferred that »Es entspricht also die mediane Anlage der Urmundslippe dem queren Gehirnwulst des Embryo«. ROUX also found that an injury to the equatorial region of the egg gave a defect in the middle of the medullary folds, while a puncture in the centre of the black hemisphere produced an extra-ovate that later appeared on the under side of the embryo. The conclusions that he drew from his experiments were that the dorsal lip of the blastopore travels about 170° over the yolk to meet the stationary ventral lip and that the embryo is formed entirely on the white hemisphere by a process of concrescence.

SCHULTZE (8) believing that normal development might be interfered with by such operations, observed the position of natural marks on eggs of Rana during the period of gastrulation and came to the conclusion that »Eine Verschiebung des Urmundes über die Unterseite des Eies findet nicht statt«. He concluded that the embryo forms entirely on the black hemisphere.

Later workers in experimenting on the egg of Rana have found that their results do not agree with those obtained by ROUX and

SCHULTZE. MORGAN, from his experiments on Rana palustris, came to the conclusion that there is an overgrowth of the white cells by the dorsal lip of the blastopore and that the embryo forms partly on the white partly on the black hemisphere by a process of concrescence. He found that an extra-ovate produced in the centre of the black hemisphere at the beginning of gastrulation is later found on the ventral surface of the embryo, and that a puncture above the dorsal lip of the blastopore when the latter has just formed, produces an extra-ovate that later appears in the cephalic region of the embryo. MORGAN'S results show also, that there is a forward movement of the ventral lip of the blastopore and that the blastopore lips close in at nearly equal rates from all sides. My results on Bufo agree in most respects with those of MORGAN, the main differences being that I find the anterior end of the Bufo embryo extending farther up on the black hemisphere than seems to be the case in the embryo of Rana palustris, and that the dorsal lip of the blastopore in the egg of Bufo moves over somewhat more of the surface of the lower hemisphere than is the case in Rana.

In 1895, EYCLESHYMER (2) published the results of a series of experiments on the egg of Amblystoma. He punctured the egg in the centre of the black hemisphere in early and late segmentation stages and found that in 7 eggs the extra-ovate later occupied a position near the median line of the embryo just beyond the transverse portion of the neural folds (agreeing with my Experiment 1). In 5 other eggs the extra-ovate appeared within the neural folds. With such a result, EYCLESHYMER states that the inference from the experiment is that the apical pole of the egg corresponds to the later head region of the embryo! This conclusion does not seem to me to be justified by the experiment. It is evident that there must have been a considerable amount of variation in the location of the punctures when the operations were made, but if one judges solely by the results the more obvious conclusion from them appears to me to be that the anterior end of the embryo does not extend up to the pole of the black hemisphere.

In a more extended series of experiments on the eggs of Acris, Amblystoma and Bufo published in 1898, EYCLESHYMER (3) describes one experiment where 10 eggs of Bufo were punctured in the following manner: one extra-ovate was produced at the apex of the black pole and another just above the equatorial zone in a line with the middle of the dorsal lip of the blastopore. In the 7 eggs that developed the lower extra-ovate was in every case in the posterior third of the neural folds, while the extra-ovate which had presumably been produced at the pole of the black hemisphere occupied different positions in different eggs, varying from far outside the neural folds in front to near the middle of the embryo. EYCLESHYMER concludes from this experiment alone that the anterior end of the embryo of Bufo lentiginosus extends up to the apex of the black pole, thus bringing it into agreement with his conclusions regarding the formation of the cephalic region in the embryo of Acris and Amblystoma. In discussing the results of his experiments, EYCLESHYMER states: »The primary area of cell activity at the upper pole of the amphibian egg forms the basis of the cephalic end of the embryo. The secondary area of cell activity on the blastopore side of the egg, forms the basis of the greater portion of the posterior half of the embryo. These two areas constitute an embryonic tract from which arises at least the anterior two thirds of the embryo. — The greater portion of the embryo arises in the darker hemisphere by differentiation in situ, and not by concrescence.« My own experiments do not lead me to agree with the above statement as I consider that the embryo of Bufo is formed fully as much on the white as on the dark hemisphere and that the cephalic portion of the embryo is formed from the material about half way between the place where the dorsal lip ot the blastopore first appears and the centre of the black hemisphere.

In 1894, ASSHETON made a few experiments on the frog's egg which were similar to those made by MORGAN. He also came to the conclusion that the embryo forms partly on the white partly on the dark hemisphere but considers that the apparent overgrowth of the dorsal lip of the blastopore is but 60°—70° while MORGAN and WILSON believe that the dorsal lip moves about 120°. My own experiments and observations lead me to think that in the egg of Bufo the dorsal lip of the blastopore moves over about 140° of the lower hemisphere of the egg.

BERTACCHINI (11), in a recent paper which I have not seen, brings forward experimental evidence to show that the dorsal lip of the blastopore moves over more than 180° of the surface of the egg.

The most recent work in this field is that of WILSON who made use of the eggs of chorophilus feriarum and Amblystoma in his experiments. WILSON found a considerable variation in his results in many cases and in consequence he is inclined to consider that more knowledge regarding the early development of the egg can be acquired through observations on the living egg than by any experimental means. The conclusions he drew from his experiments were in accord with his observations on the living egg, and in the main agree with those I obtained on Bufo. In a later paper WILSON (10) lays great emphasis on the forward movement of the ventral lip of the blastopore. He states that the »ventral lip is far from stationary, in that directly upon its formation it begins to overgrow the yolk at about the same rate as the dorsal lip«, and he estimates that the ventral lip overgrows the yolk about $47^{1}/_{2}°$. This measurement is much larger than that computed by other investigators and is certainly considerably greater than either experiments or observations would warrant my stating for the egg of Bufo.

Summary.

1) The dorsal lip of the blastopore in the egg of Bufo lentiginosus moves over the yolk from a point below the equator of the egg where it first appears, to beyond the centre of the white hemisphere (Experiment 2). The backward movement of the dorsal lip of the blastopore is still further indicated by Experiments 3, 5, 6, and 11.

2) There is a movement of the cells just above the middle of the dorsal lip of the blastopore to the edge of the blastopore (Experiments 4, 6, 22); but somewhat farther forward the cells are not involved in this movement (Experiment 5). Material also moves in to the blastopore rim from the immediate vicinity of the sides of the dorsal lip (Experiment 9) and from the sides of the lateral lips (Experiment 17), but farther away there is no movement of the cells (Experiments 10, 12).

3) Material from the equatorial region of the egg moves in to the middle line to help form the mid-dorsal portion of the embryo by a process of concrescence (Experiments 11, 17).

4) The material a little above the dorsal lip of the blastopore forms the middle portion of the embryo (Experiment 5). The anterior end of the embryo does not extend up to the pole of the black hemisphere (Experiments 1, 8), but is formed about half way between the place where the dorsal lip of the blastopore first appears and the centre of the black hemisphere (Experiment 7). The embryo, therefore, forms partly on the black and partly over the white hemisphere.

5) The ventral lip of the blastopore forms at the edge of the white area directly opposite the dorsal lip (Experiment 13). There is a forward movement of the cells in the immediate vicinity of the ventral lip (Experiments 18, 20, 21), but, the cells further away from the ventral lip are not involved in this movement (Experiments 15, 18, 19, 21).

6) The ventral lip of the blastopore moves forward over the yolk but only a short distance as compared with the movement of the dorsal lip (Experiments 18, 19, 20).

7) The region of cell activity is more extensive at the dorsal lip of the blastopore than at the ventral lip (Experiment 22).

8) The rate of movement of the cells above the middle of the dorsal lip of the blastopore is greater than that of the cells at the sides (Experiment 22). Experiment 21 indicates that the same may be true of the cells below the ventral lip.

9) After the blastopore has become circular, it closes in at nearly equal rates from all sides (Experiments 16, 23).

Bryn Mawr College, Bryn Mawr, Pa., Dec. 2nd 1901.

Zusammenfassung.

1) Die dorsale Blastoporuslippe im Ei von Bufo lentiginosus bewegt sich über den Dotter hin von einem Punkte unterhalb des Eiäquators aus bis jenseits des Centrums der weißen Hemisphäre (Versuch 2). Die Rückwärtsbewegung der dorsalen Blastoporuslippe findet sich noch weiter verfolgt in den Versuchen 3, 5, 6 und 11.

2) Es findet dabei eine Bewegung der Zellen gerade oberhalb des Centrums der dorsalen Blastoporuslippe nach dem Blastoporusrande hin statt (Versuch 4, 6, 22); aber weiter vorwärts betheiligen sich die Zellen nicht an dieser Bewegung (Versuch 5). Es verschiebt sich auch Material nach innen zum Blastoporusrande aus der unmittelbaren Nachbarschaft der Seitenpartien der Dorsallippe (Versuch 9) und von den Seiten der seitlichen Lippen her, in größerer Entfernung finden dort jedoch keine Zellverschiebungen statt (Versuch 10, 12).

3) Bildungsmaterial von der Äquatorialregion des Eies bewegt sich gegen die Mittellinie hin, um sich an der Bildung der mittleren Rückenpartie des Embryos unter Verwachsungsvorgängen zu betheiligen (Versuch 11 und 17).

4) Das Material etwas oberhalb der dorsalen Blastoporuslippe bildet die Mittelpartie des Embryos (Versuch 5). Sein vorderes Ende reicht nicht bis zum Mittelpunkt der schwarzen Hemisphäre herauf (Versuch 1, 8), bildet sich vielmehr etwa mitten zwischen der Stelle des ersten Auftretens der dorsalen Blastoporuslippe und dem Mittelpunkt der schwarzen Eihälfte (Versuch 7). Der Embryo entsteht daher theils im Gebiete der dunklen, theils an der Oberfläche der hellen Eihälfte.

5) Die ventrale Blastoporuslippe entsteht an dem der dorsalen Lippe gegenüber liegenden Rande des weißen Feldes (Versuch 13). In unmittelbarer Nachbarschaft der ventralen Lippe rücken dabei die Zellen vorwärts (Versuch 18, 20, 21); jedoch betheiligen sich die Zellen in größerer Entfernung von der ventralen Lippe nicht an dieser Bewegung (Versuch 15, 18, 19, 21).

6) Die ventrale Blastoporuslippe rückt über den Dotter gleichfalls vor, im Vergleich zur Verschiebung der Dorsallippe aber nur eine kurze Strecke weit (Versuch 18, 19, 20).

7) Der Bezirk der aktiven Zellthätigkeit ist an der Dorsallippe größer als an der ventralen (Versuch 22).

8) Der Verschiebungsbetrag ist für die Zellen oberhalb des Dorsallippencentrums größer als für die Zellen an den Blastoporusseiten (Versuch 22). Versuch 21 zeigt, dass dasselbe auch für die Zellen unterhalb der ventralen Lippe gilt.

9) Nachdem der Blastoporus cirkular geworden ist, rücken bei seinem Schluss von allen Seiten her etwa gleiche Beträge nach innen vor (Versuch 16, 23).

Literature.

1) Assheton, R., On the Growth in Length of the Frog Embryo. Quart. Journ. micr. Sci. Vol. XXXVII. 1894.

2) Eycleshymer, A. C., The Early Development of Amblystoma with Observations on Some Other Vertebrates. Journ. Morph. Vol. X. 1895.

3) —— The Location of the Basis of the Amphibian Embryo. Journ. Morph. Vol. XIV. 1898.

4) Morgan, T. H., and Tsude, Ome, The Orientation of the Frog's Egg. Quart. Journ. micr. Sci. Vol. XXXV. 1893.

5) Morgan, T. H., The Formation of the Embryo of the Frog. Anat. Anz. Bd. IX. 1894.

6) —— The Development of the Frog's Egg. The Macmillan Company. New York 1897.

7) Roux, W., Über die Lagerung des Materials des Medullarrohres im gefurchten Froschei. Anat. Anz. Bd. III. 1888.

8) Schultze, O., Über die Entwickelung der Medullarplatte des Froscheies. Verhandl. d. physik.-medic. Gesellsch. zu Würzburg. Bd. XXIII. 1889.

9) Wilson, H. V., Formation of the Blastopore in the Frog Egg. Anat. Anz. Bd. XVIII. 1900.

10) —— Closure of Blastopore in the Normally Placed Frog Egg. Anat. Anz. Bd. XX. 1901.

11) Bertacchini, P., Morfogenesi e Teratogenesi negli Anfibi anuri. II^a Serie: Blastoporo e organi assili dorsali dell' embrione. Internat. Monatsschrift f. Anat. u. Phys. 1899. Bd. 16. Heft 11/12.

THE

AMERICAN NATURALIST

A MONTHLY JOURNAL DEVOTED TO THE
NATURAL SCIENCES IN THEIR
WIDEST SENSE

REPRINT

FROM

VOL. XXXVI, NO. 427. JULY, 1902

BOSTON
GINN & COMPANY
The Athenæum Press
1902

THE GASTRULATION OF THE EGG OF BUFO LENTIGINOSUS.

HELEN DEAN KING.

An examination of the literature dealing with the early development of the amphibian egg shows many conflicting observations and theories regarding the origin of the blastopore and the manner of formation of the mesoderm and notochord. It is evident, therefore, that more forms must be studied and the observations in some cases carefully repeated before any general conclusions regarding the origin of these structures can be drawn for the entire group.

The amphibian eggs that have been most studied are those of Rana, Triton, Axolotl, and Bombinator. The present paper deals with the early development of the egg of *Bufo lentiginosus* from the end of cleavage until the closure of the blastopore. The eggs of this amphibian, although very abundant and easily obtained, have been but little used either for observation or for experiment, owing, doubtless, to their small size and to their relatively deep pigmentation, which makes it very difficult to follow the fate of living cells.

METHOD.

In preparation for sectioning, eggs were killed in various fluids: picro-acetic, picro-sulphuric, formalin, and corrosive-acetic. By far the best results were obtained with corrosive-acetic (5 per cent acetic). Formalin (3–10 per cent) gives exceedingly good preparations for a study of surface structures, but it cannot be relied on to give histological details, as it usually produces numerous cracks in various parts of the egg. The sections were stained on the slide with the mixture of borax carmine and Lyon's blue, recommended in a previous paper (King, 10). With the use of this stain the nuclei appear red, the yolk blue, and the cell outlines are brought out with great clearness.

THE FORMATION OF THE BLASTOPORE.

The egg of *Bufo lentiginosus* probably contains a greater amount of pigment in proportion to its size than that of any other common amphibian. It is, therefore, very difficult to study the movements of individual cells before and during the formation of the blastopore, as has been done to some extent in the eggs of several other species of amphibians, where the pigmentation is less extensive and cell outlines can be readily determined.

In the egg of Bufo the pigmentation extends in all cases some distance below the equator, and I have frequently found eggs in which fully three-fourths of the surface was deeply pigmented before the appearance of the blastopore. Individual eggs, even from the same female, differ greatly in the amount of pigment they contain. As a rule, the pigment line extends farther down on one side of the egg than on the other, as seen in Fig. 1, agreeing in this respect with the frog's egg according to Schultze (24), Morgan and Tsuda (17), and Wilson (27).

Sections through an egg at the close of the blastula stage show a large segmentation cavity in the upper hemisphere. Its dorsal wall is formed, as in the frog, of three or four layers of small angular cells of uniform size. The cells forming the outer surface of the egg are almost completely filled with pigment granules, and a considerable amount of pigment is scattered throughout all the cells of the upper part of the egg. The yolk cells below the segmentation cavity are much larger, more rounded, and stain less intensely than the cells in the upper hemisphere. There is the same gradual increase in the size of the cells from the upper to the lower pole that other investigators have noted in the frog's egg.

The dorsal lip of the blastopore invariably appears some distance below the equator of the egg, but never in the middle of the lower hemisphere, as maintained by Houssay (8) for the axolotl and Jordan (9) for the newt.

If the egg has but the usual amount of pigment, the first evidence of the blastopore, in surface view, is a short, dark, almost straight line at the extreme edge of the black cells (Fig. 1). If the pigmentation is unusually extensive, the blastopore appears in its same relative position with respect to the lower pole of the egg but distinctly within the black cells. Whether there is, as the first step in the formation of the blastopore, a "lining up" of the ectoderm cells, as described by Wilson for the frog, I have not been able to determine.

Sections of an egg at the beginning of gastrulation show that the dorsal lip of the blastopore is formed primarily by a sinking in of several of the surface cells (Fig. 9). The shallow depression thus formed is rapidly extended and soon becomes a pronounced furrow (Fig. 10). The cells involved in this sinking in are all, without question, large yolk cells which are decidedly wedge-shaped and contain a considerable amount of pigment in their smaller ends turned towards the exterior (Figs. 9, 10, 11).

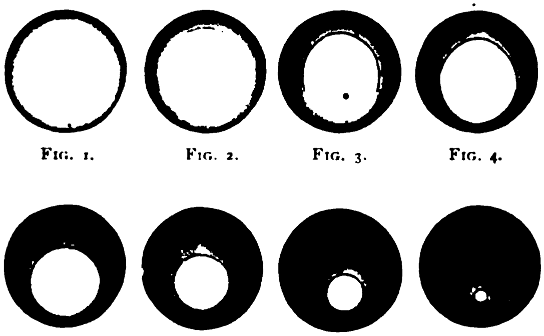


Fig. 1. Fig. 2. Fig. 3. Fig. 4.

Fig. 5. Fig. 6. Fig. 7. Fig. 8.

Figs. 1-8. — Surface views of the lower hemisphere of an egg during the formation and closure of the blastopore.

After the lateral extension of the dorsal lip to form a crescent, a sagittal section through the blastopore shows that the furrow has deepened considerably and that its inner end is turned up towards the dark pole (Fig. 11). From this time on there is a marked difference in appearance between the cells forming the dorsal wall of the archenteron and those forming the ventral wall. The cells of the dorsal wall are small, angular, deeply pigmented cells, and they are apparently exactly like the small cells forming the upper surface of the egg, except that they do not contain quite as much pigment. The yolk cells which form the floor of the archenteron are three or four times larger than the cells of the dorsal wall, and they are more rounded and contain little if any pigment. The distinction between these cells is made more evident by the fact that with the combination stain used all the yolk cells appear decidedly blue, while the cells of the dorsal wall of the archenteron and, in fact, all the cells of the upper hemisphere, take a distinctly reddish tint. The cells at the anterior end of the archenteron are still wedge-shaped at this stage. They are intermediate in size between the cells forming the dorsal wall and those forming the ventral wall of the archenteron, and they stain like the yolk cells.

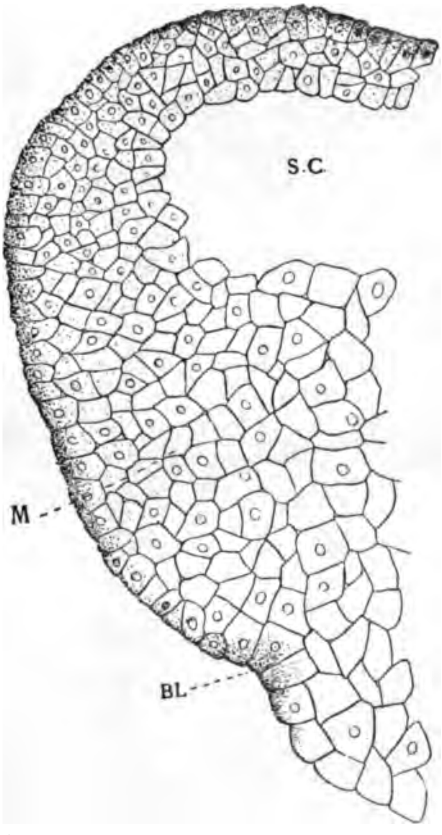


FIG. 9. — Part of a section through the median plane of the blastopore at the stage of Fig. 1, showing the depression of the yolk cells to form the dorsal lip. *S.C.*, segmentation cavity; *BL.*, blastopore; *M.*, cells which are to develop into mesoderm.

In opposition to most investigators, Moquin-Tandon (15), Houssay, Robinson and Assheton (20), and Marshall (14) maintain that the archenteron of the amphibian embryo is not formed by a process of invagination, but by a splitting between yolk cells which thus form the dorsal as well as the ventral wall of the archenteron. In a later paper, Assheton (1) states that "the formation of the primitive archenteron is by a process of splitting, and is the direct effect of the primary center of growth; whilst the continuation of the cavity produced by an overgrowth is the direct effect of the secondary center of growth."

That the archenteron in the egg of *Bufo lentiginosus* is not formed by a splitting between yolk cells is shown, I think, by a study of a series of well-preserved eggs during the early stages of gastrulation. The archenteron never appears "slit-like" at this time. On the contrary, its walls are usually some distance apart and its inner end is invariably rounded (Figs. 10, 11). Occasionally at the stage of Fig. 12, never earlier, I have seen an irregular cleft between the yolk cells at the anterior end of the archenteron which might, perhaps, be considered a forward extension of the archenteron, but such a cleft is usually seen only in badly preserved eggs where the cells are all more or less separated, and therefore I have always considered that it was artificially produced by the method used in killing and hardening the egg.

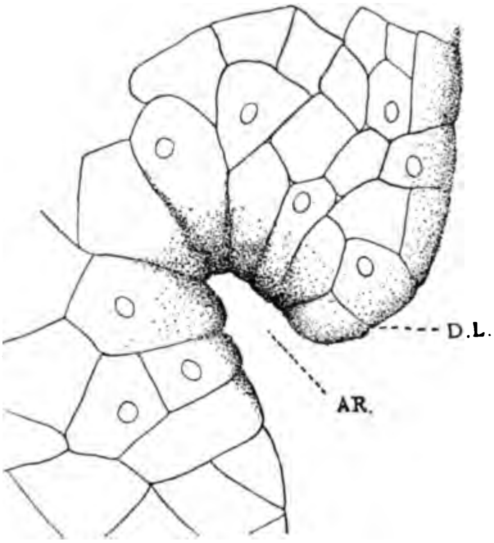


FIG. 10. — Part of a median section through the blastopore at a slightly later stage than Fig. 9, showing the character of the cells at the base of the blastopore furrow. *D.L.*, dorsal lip of the blastopore; *AR.*, beginning of the archenteron.

Jordan, Wilson, and Eycleshymer (4), among others, have watched the disappearance of individual surface cells under the dorsal lip of the blastopore in the early gastrulation stages of the living egg. These observations seem to me to afford conclusive evidence that invagination of cells plays an important rôle in the formation of the archenteron.

The presence of pigment in the inner ends of the cells forming the dorsal wall of the archenteron has been explained by Robinson and Assheton as follows: "The pigmented area is produced and extended by the deposit of pigment in the adjacent margins of a double row of yolk cells which eventually will form the boundary wall of the archenteron, and it also radiates from this area along the adjacent margins of the cells of each row. A slit-like space appears in the middle of the posterior portion of the pigmented area. This space first limits the dorsal lip of the blastopore, and then extends forward and ventrally, following the deposit of pigment, and separating the two rows of marginally pigmented cells from each other." During the early stages of gastrulation there is always found at the anterior end of the archenteron a marked accumulation of pigment that is extended around to include a few of the yolk cells in this region (Figs. 10–12). I have never found more than a few scattered pigment granules in the lower yolk cells except in this particular part of the egg, and, if the archenteron is formed by a splitting

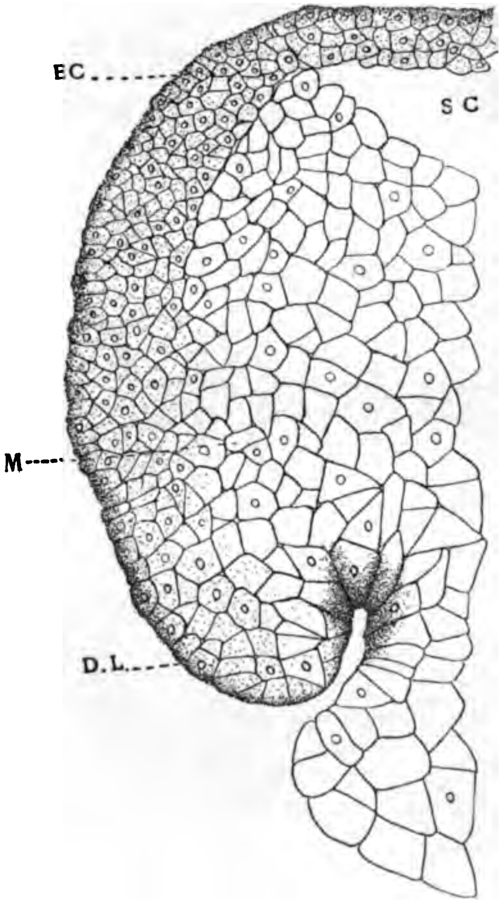


FIG. 11. — Part of a median sagittal section through the blastopore at the stage of Fig. 2. *EC.*, ectoderm. Other lettering as in previous figures.

between marginally pigmented yolk cells, then the pigment must at once almost entirely disappear on one side of the split and not on the other.

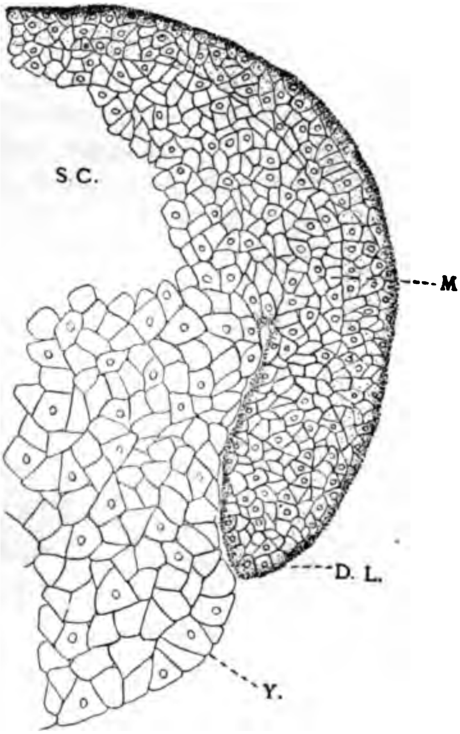


Fig. 12. — Part of a median sagittal section through the blastopore at the stage of Fig. 3. Y., yolk plug. Other lettering as in previous figures.

The pigmentation at the anterior end of the archenteron in the egg of Bufo is not confined to "a double row of yolk cells" anterior to the actual cleft. Well-marked lines of pigment may extend out in any direction, even at right angles to the line of advance of the archenteron, as seen in Fig. 13. From the time that the blastopore is first formed, the pigment in the cells of the dorsal wall of the archenteron is always collected around the cell walls, never around the nucleus. Rhumbler (22) considers this phenomenon to be due to the mechanical effect of pressure. The suggestion has been offered by Jordan "that the pigment marks physiological activity, and that the less heavily pigmented cells of the ventral wall of the archenteron owe their relative lack of pigment to more sluggish metabolism attendant upon less rapid cell division." It is certainly true that the large yolk cells in the egg of Bufo divide less frequently than the cells in the upper hemisphere, but there is no evidence that the deeply pigmented cells of the outer surface of the upper hemisphere or of the dorsal wall of the archenteron divide more rapidly than the cells that are found between them. If, therefore, Jordan's suggestion is correct, some kind of "physiological activity" other than that attendant upon rapid cell division must produce the pigmentation in these cells.

The extension of the lateral lips of the blastopore and the formation of the yolk plug have been so frequently described for other forms that a description of these processes is not necessary here, as they are apparently similar in all respects to those which take place in the frog. Following the method used by Pflüger (20), a number of eggs in the blastula stage were placed on a mirror in a shallow dish of water and the formation of the blastopore watched in mirror image. Figs. 1–8 show the appearance and the location of the blastopore at different times during the day, from 8 A.M., when the blastopore lip first appeared, to 6 P.M., when the egg began a rotation around its horizontal axis. There was no evidence to show that the position of the eggs was changed during the time they were kept under observation; on the contrary, one egg, in which a peculiar light spot was seen below the equatorial region, was watched particularly, and the spot was found to keep its same relative position until the rotation of the egg took place. These mirror images show that the dorsal lip of

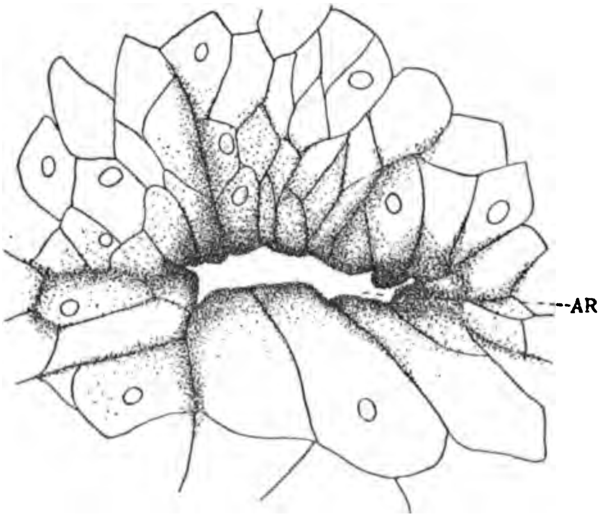


FIG. 13. — Part of a frontal section through the anterior end of the archenteron at the stage of Fig. 3, showing lines of pigment radiating out in all directions. *AR.*, archenteron.

the blastopore appears below the equator of the egg at the edge of the black cells (Fig. 1), and that the blastopore rim extends at a uniform rate in the form of a crescent around the lower hemisphere, until finally the yolk plug is formed (Figs. 2–4). Meanwhile, the dorsal lip of the blastopore has moved over the yolk, and seven hours after its first appearance has reached the center of the white hemisphere (Fig. 6). The closure of the blastopore lips takes place almost uniformly from all sides as a rule (Fig. 7), although occasionally the blastopore is somewhat oval when it is in the stage of Fig. 8. At or just before the stage of Fig. 8 a rotation of the egg around its horizontal axis takes place, thus bringing the dorsal lip of the blastopore back to its original position.

In its movement over the lower hemisphere the dorsal lip of the blastopore seems to pass through about 140° of the surface of the egg. There is apparently a slight variation in this respect in different eggs. This measurement is somewhat greater than that given by Morgan (16) and by Wilson (27) for the movement of the dorsal lip of the blastopore in the frog egg, and differs considerably from the measurements which have been given by other investigators. Roux (21) and Pflüger (19) estimate that the dorsal lip moves about 170°, while Kopsch (11) considers the movement to be but 75°; and this figure is further reduced by Assheton, who states that much of the so-called movement of the dorsal lip is only apparent and that the real movement is only from 60° to 70°.

THE FORMATION OF THE "GERM LAYERS" AND THE NOTOCHORD.

Observations regarding the origin of the endoderm, mesoderm, and notochord in different amphibian eggs are so conflicting that apparently the most plausible interpretation of them is to assume that there is absolutely no uniformity in the manner of formation of these structures even in species that are considered to be very nearly related to each other. Such an assumption, however, can scarcely be the correct one.

Goette (6), one of the early workers on the development of the amphibian egg, considered the mesoderm in the egg of Bombinator to arise from the splitting off of a layer of cells from the primitive entoblast, the layer thus formed extending as an unbroken sheet across the dorsal wall of the archenteron. The rest of the primitive entoblast was said to form the endoderm. According to Goette, a central chord of mesoderm in the mid-dorsal region of the embryo separates from the two lateral sheets to form the notochord.

A few years later Hertwig (7), after studying the development of the eggs of Triton and of *Rana temporaria*, came to the conclusion that the mesoderm in the amphibian egg arises in the vicinity of the blastopore "durch eine paarige Einfaltung des Entoblast schon zu einer Zeit wo die Gastrulaeinstülpung noch nicht ganz vollendet ist." The amphibian egg was thus brought into agreement with Hertwig's observations on the development of other vertebrates and gave additional support to his well-known cœlom theory. A few other writers — Balfour (2), Marshall (14), and Schwink (26) — agree with the results obtained by Hertwig.

In 1888, Schultze (25), from observations on *Rana fusca*, decided that "das mittlere Blatt, sowie die dorsale Urdarmwand entstehen aus dem Ektoblast und gehen an der dorsalen Urmundlippe alle drei Blätter in einander über; in den seitlichen und ventralen Theilen des Blastoporus setzt sich die Deckschicht des äusseren Keimblattes mit besonderer Klarheit in den Entoblast, die Grundschicht des Ektoblast ohne Unterbrechung in den Mesoblast fort." Perenyi (18), from observations on the egg of *Bombinator igneus*, Lwoff (13), from a study of Axolotl and various Anura, and Brauer (3), from investigations on two species of Gymnophiona, also came to the conclusion that the mesoderm is ectodermal in origin. These investigators, however, differ somewhat regarding the manner in which this process is supposed to take place.

Perenyi states that, as a result of a turning under, or "Duplikation," of the three-layered outer wall of the egg at the lips of the blastopore, "die äussersten Deckzellen der Blastula in ihrer ununterbrochenen Fortsetzung nach innen endlich die untersten

Zellenreihen bilden werden d. h. sie verwandeln sich in Entoderm, während die anderen zwei Zellenreihen der Blastula auch in ihrer Rückwindung beisammen bleiben und eine selbständige Schichte, das Mesoderm, bilden."

According to Lwoff, "die Einwanderung der Ektodermzellen und die Verschiebung der Entodermzellen zwei verschiedene und von einander unabhängige Vorgänge sind. Die Einwanderung von Ektodermzellen beginnt an der Stelle die das Hinterende des Embryo markirt; die Verschiebung der Entodermzellen beginnt im Gegentheil im vorderen Theile. Wie die weiteren Stadien lehren, bildet das Hineinwachsen der Ektodermzellen die zusammenhängende ektoblastogene Anlage der Chorda und des Mesoderms; die Verschiebung der Entodermzellen führt zur Bildung der Darmhöhle, die infolge des Auseinanderweichens der Entodermzellen entsteht."

Brauer's investigations show that there is a turning under of the outer surface cells, or "animal cells," at the posterior end of the germ disk and that by the forward growth of these cells under the upper animal cells a blind sac is formed. This blind sac becomes the posterior part of the archenteron, and at first its dorsal wall is composed of the animal cells invaginated from the surface. In subsequent development a layer of vegetative or yolk cells grows up from either side of the archenteron and forms a layer of endoderm beneath the invaginated animal cells which later become the mesoderm.

The origin of the mesoderm in the frog's egg has been described by Morgan (16) as follows: "The cells that are to form the mesodermal layer are present at the time when the dorsal lip of the blastopore has first appeared, and even just prior to that time." The innermost of the cells forming a ring around the equatorial region of the egg where the black and the white cells meet are the cells that later become the mesoderm. "These cells are carried up to the median dorsal line of the embryo by the closure of the blastopore. They will then be found forming a layer or sheet of cells that separates itself on the outer side from the thick layer of small ectodermal cells (that has been simultaneously lifted up) and that is separated on the inner surface, but not very sharply if at all, from the dorsal and dorsal-lateral walls of the archenteron."

In the egg of *Bufo lentiginosus*, when the blastopore first appears as a slight depression among the yolk cells just below the equator of the egg (Fig. 9), the cells which form the dorsal wall of the segmentation cavity are all small, somewhat angular cells which contain a considerable amount of pigment, particularly the cells which compose the outer surface of the egg. Near the equator the cells are found to be somewhat larger and to contain much less pigment, while in the lower hemisphere the cells are very large, and they have but a few scattered pigment granules. Up to this stage of development there is no visible separation of the cells into ectoderm, endoderm, and mesoderm. The smaller cells resulting from rapid cell division are found in the upper part of the egg and the larger cells, which divide less frequently, are grouped around the lower pole. The mass of cells marked *M* in Fig. 9 undoubtedly represent the region of the egg from which the mesoderm is formed. These cells are in no wise distinctive at this period, and their later development into mesoderm I consider to be due solely to their position in the egg during the formation and closure of the blastopore. The cells which are to become mesoderm form a layer around the egg at the equatorial region just inside the cells that are to become the ectoderm, as found to be the case in the frog's egg, according to Morgan. These mesoderm cells have many characteristics in common with the large yolk cells into which they grade, being larger, more rounded, and containing much less pigment than the small cells of the upper hemisphere. It seems probable, therefore, that they were originally produced by divisions of the yolk cells.

When the lips of the blastopore have extended so as to form a crescent in surface view (Fig. 3) a frontal section through an egg in the region of the blastopore (Fig. 14) shows that the dorsal wall of the archenteron is formed of several layers of small cells which have absolutely no distinction between them except that the cells of the outer layer, which are more regularly arranged, contain a much greater amount of pigment than the other cells. At the sides of the archenteron the innermost of these cells pass into the large yolk cells which form the floor of the archenteron. A median sagittal section through the blastopore at the same or a slightly earlier stage of development (Fig. 11) shows the beginning of the separation of the ectoderm from the inner cells of the egg. A tolerably regular cleft extends some distance around the sides of the egg, on a line usually with the lower edge of the dorsal wall of the segmentation cavity, sharply separating the layer of cells forming the outer wall of the egg from the cells within. The separation of the ectoderm does not extend as far down as the equator of the egg at this time, and it is some hours later before the ectoderm at the lips of the blastopore is distinct from the other cells. It is in the region of the blastopore that the union of the different layers persists longest, as other investigators have noted.

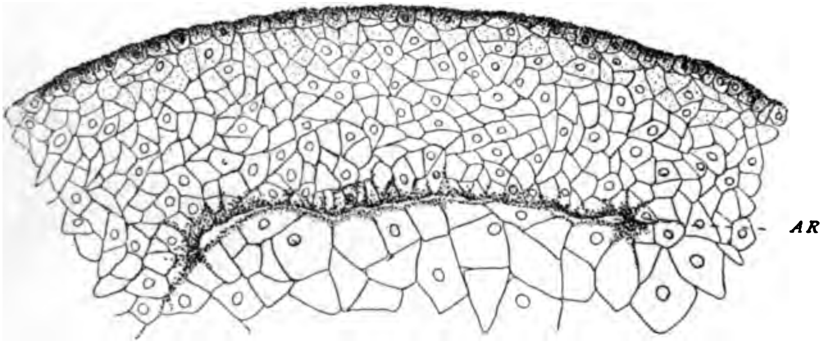


FIG. 14. — Part of a frontal section through the region just anterior to the blastopore at the stage of Fig. 3. *AR.*, archenteron.

At the stage of Fig. 3 the formation of the archenteron is well advanced, and a median sagittal section through the blastopore (Fig. 12) shows that the endoderm of the dorsal wall of the archenteron is formed of small, angular, deeply pigmented cells which, as far as I am able to determine, appear exactly like the small cells which form the outer surface of the egg. It seems probable that these cells once formed a part of the outer surface of the egg in the region just outside of the blastopore, and that they have been turned under the edge of the blastopore lip by a process of invagination, thus being changed into endoderm. Whether, in later stages of development, the endoderm of the dorsal wall of the archenteron receives additions from the yolk, as believed to be the case by Assheton and by Wilson, I have not been able to determine.

After the ventral lip of the blastopore has formed and the blastopore is beginning to close (Fig. 6), a division of the ectoderm into two layers is first noticed. A median sagittal section at this period (Fig. 15) shows an outer ectodermal wall composed of a single layer of small, deeply pigmented cells which are arranged very regularly. The inner ectodermal sheath is composed of two or three layers of much larger cells

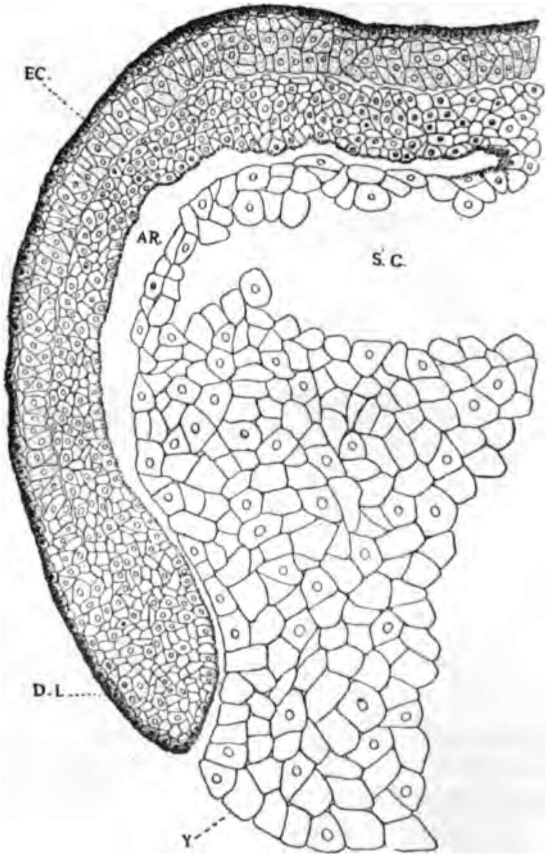


FIG. 15. — Part of a median sagittal section through the egg at the stage of Fig. 6. Lettering as in previous figures.

which are distinctly wedge-shaped and contain a comparatively small amount of pigment. The inner layer of ectoderm is sharply separated from the mass of small cells above the dorsal wall of the archenteron.

When, in surface view, the blastopore has reached the stage of Fig. 6, or in some cases even as early as Fig. 5, a splitting is seen in the mass of cells above the archenteron, and a single layer of endodermal cells is separated from the cells above. This process begins some distance in front of the region of the blastopore and gradually extends forward and backward from this point, thus completing the separation of the cells in the mid-dorsal region of the embryo into ectoderm, mesoderm, and endoderm.

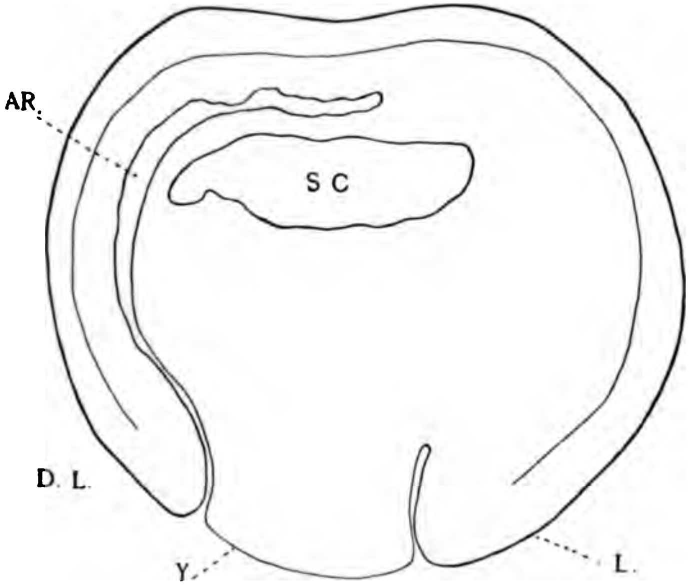


FIG. 16. — Outline of the entire section from which Fig. 15 was drawn. *L.*, ventral lip of the blastopore.

When the blastopore has nearly closed (Fig. 8), a sagittal section through the middle of the dorsal lip (Fig. 17) shows that the endoderm cells of the dorsal wall of the archenteron (Fig. 17, *En.*) have flattened out considerably, and that they still contain much pigment, which is collected in the end of the cell bordering the cavity of the archenteron. Above the endoderm is the mesoderm layer (Fig. 17, *M.*), which varies in the number of its cells in different parts of the egg. Near the dorsal lip of the blastopore the mesoderm sheath consists of several layers of small cells which are not pigmented. Forward from this region the mesoderm gradually thins out until it is composed of only a single layer of rounded cells lying between the endoderm and ectoderm; then it gradually becomes thicker again, and near the ventral lip of the blastopore it appears very much as it does near the dorsal lip, being composed of several layers of cells.

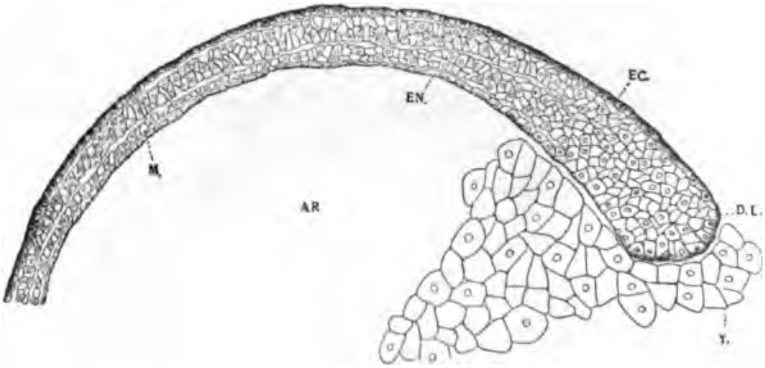


Fig. 17.—Part of a median sagittal section through the egg at the stage of Fig. 8. *E.N.*, entoderm. Other lettering as in previous figures.

A frontal section through the middle of an egg at the stage of Fig. 7 shows a single layer of mesoderm over the dorsal region of the embryo, which gradually becomes several layers thick at the sides of the archenteron (Fig. 19), and below the archenteron passes directly into the yolk cells of the lower part of the egg. Still later stages during the closure of the blastopore show that the mesoderm is unquestionably extended at the expense of the yolk cells lying just beneath the ectoderm. Soon after the medullary folds have appeared the lateral sheets of mesoderm become fused on the ventral side of the embryo; thus a continuous sheet of mesoderm is formed around the embryo, except in the mid-dorsal region where the notochord has by this time been cut off from it. The extension of the lateral sheets of mesoderm by a process of splitting off from the outer yolk cells has been noted by Schwink (26) in *Bufo vulgaris*, by Scott and Osborn (23) and by Jordan in the newt, and by Morgan in the frog.

At the close of the blastula stage a very large segmentation cavity is found in the upper hemisphere of the egg directly under the black pole (Figs. 9, 11, 12). During the formation of the archenteron this segmentation cavity decreases considerably in size, and is pushed out of its position, coming to lie below the archenteron and being separated from it by only a thin layer of cells (Fig. 16, *S.C.*). During the closure of the blastopore the archenteron increases greatly in size, and there is then usually but a single layer of large yolk cells between it and the segmentation cavity (Fig. 19). When the blastopore is nearly closed, I have frequently found eggs in

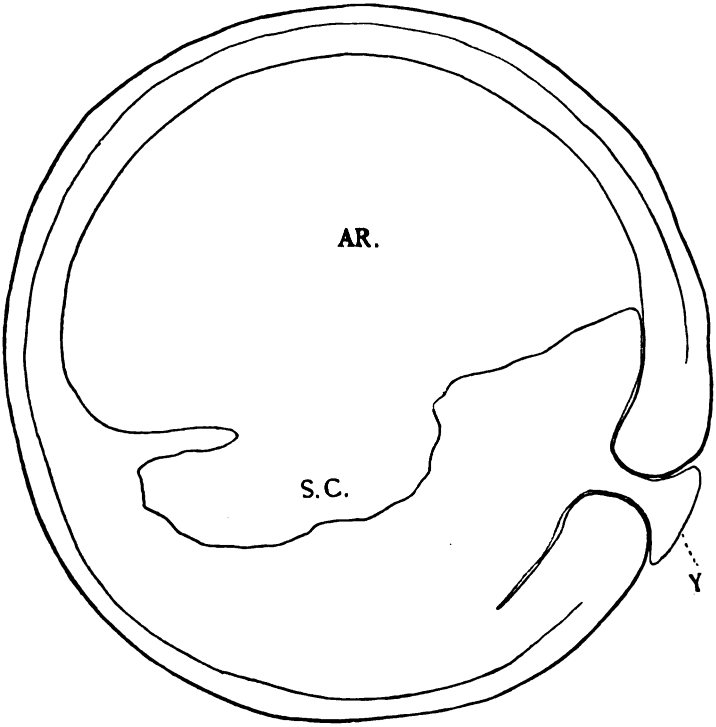


FIG. 18. — Outline of the entire section from which Fig. 17 was drawn.

which there appeared to be a direct connection between the two cavities which could not be due to poor preservation of the material (Fig. 18). It would seem, therefore, that in some cases the archenteron is extended at the expense of the segmentation cavity, as supposed to be the case by Kupffer (12) and by Marshall.

A series of frontal sections through the embryo at about the stage of Fig. 7 will show the various steps in the formation of the notochord. A section just in front of the dorsal lip of the blastopore (Fig. 20) shows that the mesoderm in this region forms a continuous sheath over the dorsal wall of the archenteron and that it is composed of three to four layers of small cells (Fig. 20, *M.*). About eight sections anterior to Fig. 20 there is a noticeable thickening of the mesoderm in the mid-dorsal region (Fig. 21), while on either side of this thickening the mesoderm usually consists of but two layers of cells. More anterior, the thickened part of the mesoderm layer is completely separated from the lateral sheets

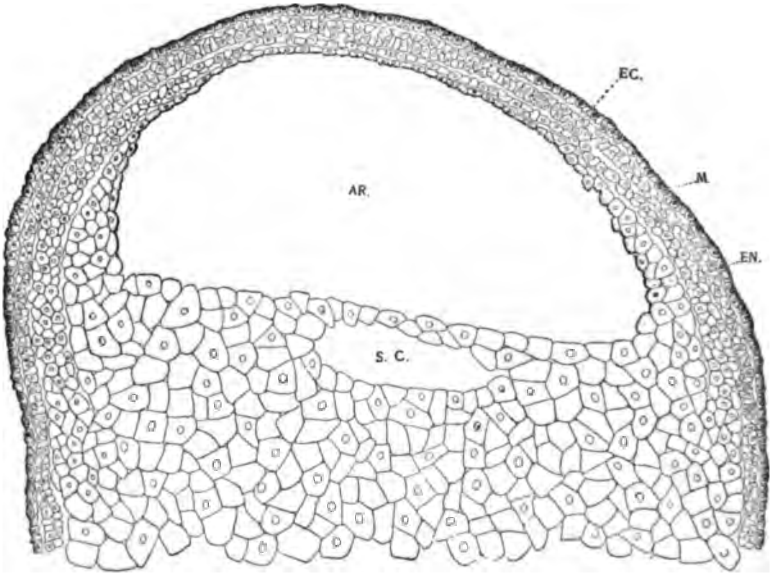


FIG. 19. — Part of a frontal section through the middle region of an egg at the stage of Fig. 7, showing the growth of the mesoderm at the expense of the yolk. Lettering as in previous figures.

as a distinct rounded structure, the notochord (Fig. 22, *N.*). For only a short distance at this stage of development is the notochord entirely cut off from the mesoderm; beyond there is the same thickening of the mid-dorsal portion of the mesoderm sheet as seen in Fig. 21. In the head region of the embryo the mesoderm is thinned out to a layer one or two cells deep which shows absolutely no thickening in the middle and appears very much as in Fig. 19. After the blastopore has closed, the notochord becomes extended forward in the head region and backward in the region of the tail.

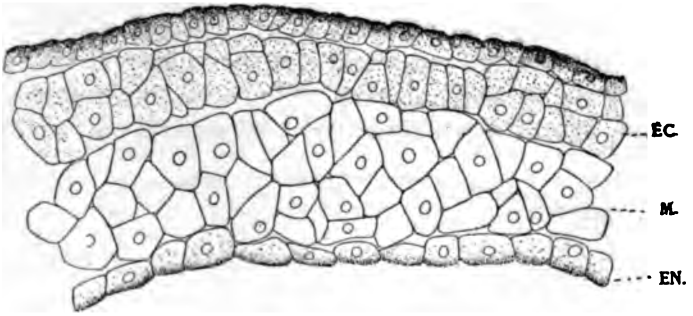


Fig. 20. — Part of a frontal section through an egg at the stage of Fig. 7, showing the mid-dorsal region of the embryo just in front of the blastopore. *EC.*, ectoderm; *EN.*, endoderm; *M.*, mesoderm.

Schultze, Goette, Lwoff, Brauer, Schwink, and Morgan also believe that the notochord is derived from the mesoderm; while other investigators consider that in the amphibians, as in other vertebrates, the notochord is endodermal in origin. Although Field (5) believes that in *Bufo vulgaris* the notochord arises

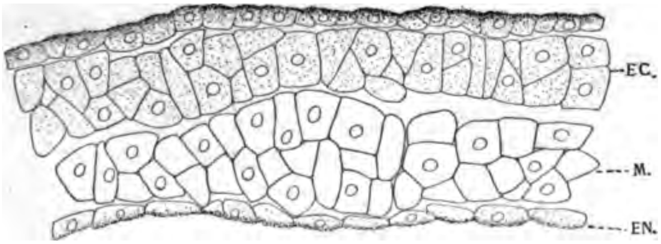


Fig. 21. — Part of a section from the same egg as Fig. 20 and slightly anterior to it. *EC.*, ectoderm; *EN.*, endoderm; *M.*, mesoderm.

from the endoderm, in the nearly related species, *Bufo lentiginosus*, the anterior part of the notochord is certainly mesodermal in origin. After the mesoderm layer has formed, the endoderm in the mid-dorsal region of the embryo is never more than a single layer of flattened cells. There is a closer connection between the mesoderm and the endoderm above the center of the archenteron than at the sides, but I have never found an egg in which these two layers could not readily be distinguished from each other. The endoderm cells are always much flattened at this period, and they invariably contain a considerable amount of pigment. The mesoderm cells and also the cells of the notochord are larger, more rounded, and contain but little pigment in comparison with the endoderm cells.

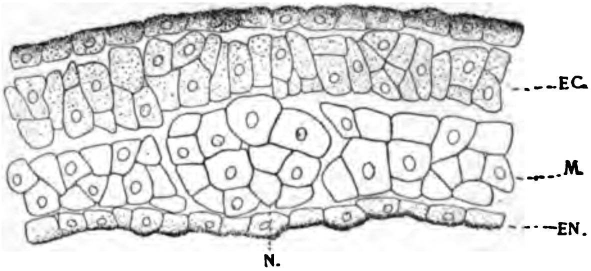


FIG. 22. — Part of a section from the middle region of the same egg. *EC.*, ectoderm; *EN.*, endoderm; *M.*, mesoderm; *N.*, notochord.

In conclusion, I wish to express my thanks to Prof. T. H. Morgan for advice and criticism during the progress of my work.

BRYN MAWR COLLEGE, BRYN MAWR, PA.,
December 16, 1901.

LITERATURE.

1. ASSHETON, R. On the Growth in Length of the Frog Embryo. *Quart. Journ. Micr. Sci.* Vol. xxxvii. 1894.
2. BALFOUR, F. M. Comparative Embryology. II. New York, The Macmillan Company, 1880.
3. BRAUER, A. Beiträge zur Kenntniss der Entwicklungsgeschichte und der Anatomie der Gymnophionen. *Zool. Jahrb.* Bd. x. 1897.
4. EYCLESHYMER, A. C. The Early Development of Amblystoma, with Observations on Some Other Vertebrates. *Journ. Morph.* Vol x. 1895.
5. FIELD, H. H. Bemerkungen über die Entwicklung der Wirbelsäule bei den Amphibien; nebst Schilderung eines abnormen Wirbelsegmentes. *Morph. Jahrb.* Bd. xxii. 1895.
6. GOETTE, A. Die Entwickelungsgeschichte der Unke. Leipzig, 1875.
7. HERTWIG, O. Die Entwicklung des mittleren Keimblattes der Wirbelthiere. I, II. Jena, 1881, 1883.
8. HOUSSAY, F. Études d'embryologie sur les vertébrés. *Arch. de Zool. Exp. et Génér.* Tome viii. 1890.
9. JORDAN, E. O. The Habits and Development of the Newt. *Journ. Morph.* Vol. viii. 1893.
10. KING, H. D. The Maturation and Fertilization of the Egg of Bufo lentiginosus. *Journ. Morph.* Vol. xvii. 1901.
11. KOPSCH, F. Beiträge zur Gastrulation beim Axolotl und Froschei. *Verh. Anat. Gesellschaft.* 1895.
12. KUPFFER, C. Die Entstehung der Allantois und die Gastrula der Wirbelthiere. *Zool. Anzeiger.* Bd. ii. 1879.
13. LWOFF, B. Die Bildung der primären Keimblätter und die Entstehung der Chorda und des Mesoderms bei den Wirbelthieren. *Bull. de la Soc. Imp. des Nat. de Moscou.* Bd. viii. 1894.
14. MARSHALL, A. M. Vertebrate Embryology. New York, Putnam, 1893.
15. MOQUIN-TANDON, G. Recherches sur les premières phases du développement des batraciens anoures. *Ann. des Soc. Nat.* Tome iii. 1876.
16. MORGAN, T. H. The Development of the Frog's Egg. New York, The Macmillan Company, 1897.
17. MORGAN, T. H., and TSUDE, UME. The Orientation of the Frog's Egg. *Quart. Journ. Micr. Sci.* Vol. xxxv. 1893.
18. PERENYI, J. Die Entwickelung der Keimblätter und der Chorda in neuer Beleuchtung. *Anat. Anzeiger.* Bd. iv. 1889.
19. PFLÜGER, E. Ueber den Einfluss der Schwerkraft auf die Theilung der Zellen. *Pflügers Archiv.* Bd. xxxii. 1883.

20. ROBINSON, A., and ASSHETON, R. The Formation and Fate of the Primitive Streak with Observations on the Archenteron and Germinal Layers of Rana temporaria. *Quart. Journ. Micr. Sci.* Vol. xxxii. 1891.
21. ROUX, W. Ueber die Lagerung des Materiales des Medullarrohres im gefurchten Froschei. *Anat. Anzeiger.* Bd. iii. 1888.
22. RHUMBLER, L. Physikalische Analyse von Lebenserscheinungen der Zelle. III. Mechanik der Pigmentzusammenhäufungen in den Embryonalzellen der Amphibieneier. *Arch. f. Entwickelungsmechanik.* Bd. ix. 1899.
23. SCOTT, W. B., and OSBORN, H. F. On Some Points in the Early Development of the Common Newt. *Quart. Journ. Micr. Sci.* Vol. xix. 1879.
24. SCHULTZE, O. Zur Entwickelung des braunen Grasfrosches. *Festschrift f. Kölliker.* 1887.
25. SCHULTZE, O. Die Entwicklung der Keimblätter und der Chorda dorsalis von Rana fusca. *Zeitschr. f. wiss. Zool.* Bd. xxvii. 1888.
26. SCHWINK, F. Ueber die Entwicklung des mittleren Keimblattes und der Chorda dorsalis der Amphibien. München, 1889.
27. WILSON, H. V. Formation of the Blastopore in the Frog Egg. *Anat. Anzeiger.* Bd. xviii. 1900.

THE

AMERICAN NATURALIST

A MONTHLY JOURNAL DEVOTED TO THE
NATURAL SCIENCES IN THEIR
WIDEST SENSE

REPRINT

FROM

VOL. XXXVI, NO. 427. JULY, 1902

BOSTON
GINN & COMPANY
The Athenæum Press
1902

HISTOLOGICAL CHANGES IN HYDRA VIRIDIS DURING REGENERATION.

HANNAH TERESA ROWLEY.

ALTHOUGH a great deal of work has been done on the regeneration of hydra, no one has as yet attempted to make out the histological changes that take place. This point would seem to be one of special interest, since the old piece appears to change its form as a whole to produce a new animal. The principal question to which I wished to find an answer was whether, during the period of regeneration, the old cells go over without change into the tissue of the new animal, or whether new cells are formed, and if so, in what part or parts.

Green hydras were used almost exclusively, since they regenerate more readily and with fewer abnormalities than does the brown species. Hydras of various lengths were taken, the foot end and circle of tentacles were cut away, and the remaining middle parts of the body wall were each divided by cross cuts into four — in some cases two — small rings. These were allowed to regenerate, and at different periods, ranging from immediately after the cutting to some days after the formation of new tentacles and foot, were killed in a solution of corrosive acetic, hardened, sectioned lengthwise, and stained in Delafield hæmatoxylin. In some cases the slides were dipped for a moment into a weak solution of picric acid in absolute alcohol, in order to differentiate the endodermal tissue.

Observation of the living piece showed that the ends became rounded, closing in from fifteen to sixty minutes after having been cut; that the piece then remained without permanent change of form for thirty or forty hours, although at intervals it might change its shape by expanding and contracting. At about this time small knoblike outgrowths, the tentacles, began to appear at one end, the other end fixed itself to the nearest support, and in a few days the proportions of a normal hydra were assumed.

Serious difficulties arose in the microscopic study of the prepared sections, which it may be well to mention. Although undoubted karyokinetic divisions were seen in both neuromuscular and interstitial cells of the ectoderm, and in the endoderm, it was found almost impossible to determine the exact amount of dividing tissue, for the following reasons.

Throughout the entire period of regeneration there is an active production of new nettle cells, which are formed from interstitial cells of the ectoderm. This process begins by a slight sickle- or moon-shaped thickening of protoplasm along a part of the cell wall. In certain sections this thickening appears as a small, darkly stained rod which may easily be mistaken for a small nucleus in process of division. Again, in sectioning, this curved, thickened rod may be cut at such an angle as to bring distinctly into view only its extremities, producing the appearance of two small groups of darkly stained material. These could be distinguished from two separating groups of chromosomes only by bringing into focus the connecting line of the rod, which would lie slightly above or below the ends. In many cases it was found almost impossible to determine whether such a cell was forming a cnidocyst or was dividing karyokinetically. These difficulties were increased by the small size of many of the interstitial cells, by the fact that the wall of such cells is not sharply defined, and by the fact that at the time of the formation of nettle cells it is very often difficult to make out the nuclei of the changing cells.

Moreover, many nuclei of both neuro-muscular and interstitial cells were found to be in a state that distinctly suggested activity of some sort, — the chromatin mass large and loose, or even in many interstitial cells broken and scattered throughout the nucleus. This appearance was very different from that of nuclei of typical resting cells in the same section, the latter containing one or two well-defined nucleoli. Yet the large number of nuclei with the chromatin scattered as just described, in sections containing comparatively few karyokinetic spindles, would seem to indicate that all such cells were not necessarily dividing. Where so many cells were about to divide or had just completed division, we should expect to find a corresponding number undergoing the actual process. This, however, was not the case.

A further point should also be kept in mind, *viz.*, the possibility that multiplication of the interstitial cells may be concerned with the development of new nematocyst cells rather than with the regeneration of new tissue.

In order to get more light on these points, the tissue of a normal hydra was examined for comparison with that of the regenerating piece. Another hydra was irritated with a blunt needle in order to induce it to discharge the nettle batteries. It was then left undisturbed for about twenty hours, at which time it was killed and the tissue prepared as has been described. It was hoped by these means to obtain further light on the difficulties of the question, and to be able to distinguish more clearly between the phenomena of regeneration and those merely incidental to the formation of new nettle cells.

In the case of the stimulated hydra, nettle cells were found to be forming as in the regenerating pieces. Moreover, in the tissue of this hydra and of the undisturbed normal one also I was surprised to find undoubted evidence of cell division, in addition to the very general loose and broken appearance of the chromatin in some of the interstitial cells, which has been noted as occurring in the regenerating piece. This latter condition, found in a hydra upon which no operation had been performed, made it still less possible to consider such nuclei as dividing. On the other hand, a piece that had been regenerating seven days, and that had attained to the proportions of a normal polyp, was found to present the same appearance under the microscope. It is possible, therefore, that the normal tissue examined was that of a growing hydra, and that these conditions are common to the regenerating and growing forms, while further examination of the tissue of fully grown hydras might show a somewhat different state.

While these latter experiments, therefore, threw little light on the amount of division in the regenerating piece, yet the similarity in appearance between this regenerating tissue, the tissue of the regenerating piece after the normal form had been attained, and the tissue of normal or growing hydra is of importance in a consideration of regeneration in this form.

I shall now attempt to make a conservative statement of the amount of dividing tissue in regenerating hydra, based on undoubted cell division.

There is very little evidence of the formation of new tissue during the early stages; an exception was noted in one piece killed five hours after the cutting, in which a number of divisions were observed in the ectoderm. Some few divisions can be observed before twenty hours, but from forty hours on, — the time of tentacle formation, — the division is more active and the number of dividing cells, especially neuro-muscular cells, is considerable. Division continues after the tentacles have begun. Spindles were seen in endodermal cells, and in neuro-muscular and interstitial cells. These divisions often occur in groups, especially in the neuro-muscular cells. In one case as many as five dividing nuclei of neuro-muscular cells were observed at one spot in a section cut from the side of the piece and so affording a surface view of ectoderm tissue alone. Fewer divisions were seen in the endoderm than in the ectoderm, partly, perhaps, because of the smaller number of cells in the endoderm.

Unlike the majority of cases of regeneration, however, the new cells are not formed at the cut surface alone, and the tentacles do not seem to be regenerated solely from new tissue. Divisions were found to occur as well at the sides as at the ends of the regenerating pieces, and in almost, if not quite, as great number. The first appearance of the tentacle, which in the living animal seems to be brought about by an outpushing of the tissue of the original piece, appeared under the microscope to be due very often to contraction in this region, causing a very slight protuberance of ectoderm, which could be fully accounted for by an outpushing of old material without the formation of new tissue. Divisions were sometimes seen at this place, sometimes not. Very frequently, however, in later stages, when the tentacle had grown to some length, there was seen to be a group of neuro-muscular cells dividing at the base of the tentacle, and interstitial cells were seen to be dividing quite rapidly in the tentacle itself. It would seem, on the whole, that new tissue is furnished to the tentacle by division of cells at the proximal end, or base, rather than by division of cells that have already entered into the new organ.

With the exception of the formation of nettle cells from interstitial cells, it is not found that cells of one kind give rise to cells of another, but that neuro-muscular cells of the new hydra result from neuro-muscular cells of the piece, and interstitial from interstitial.

Considering, therefore, that the first appearance of the tentacle may be unaccompanied by cell division in its immediate neighborhood; that cell division, though frequently found in the region of the growing tentacle, is not confined to this region but is found elsewhere throughout the length of the regenerating piece; that division is found to be present as late as seven days after the cutting, that is, after the piece has assumed the proportions of a normal hydra; and that some division is observed in the tissue of the normal, probably rapidly growing hydra, — we are justified, I think, in concluding that the new cells which appear during the regeneration of hydra are formed by division of the old cells throughout the entire piece, as in the normally growing animal, and that the tentacles are formed from old cells and from cells that have arisen by division of the already differentiated cells of the old part.

I am very much indebted to Prof. T. H. Morgan, under whose direction this work was carried on.

BRYN MAWR, January, 1902.

THE

AMERICAN NATURALIST

A MONTHLY JOURNAL DEVOTED TO THE
NATURAL SCIENCES IN THEIR
WIDEST SENSE

REPRINT

FROM

VOL. XXXVI, NO. 428. AUGUST, 1902

BOSTON
GINN & COMPANY
The Athenæum Press
1902

THE REGENERATION OF THE PHARYNX IN PLANARIA MACULATA.

HENRIETTA F. THACHER.

In a paper on *Planaria maculata*, published in 1898, Morgan called attention to an important feature of the regeneration of the pharynx, namely, that in regenerating pieces which were cut anterior to the old pharynx, the new pharynx forms "at the edge of the old tissue, but lying for the most part within the *new* material," while if the cut were made posterior to the pharynx the new organ forms in the anterior end of the *old* tissue. During the next two years there appeared a number of papers describing experiments on the power of regeneration in planarians, but giving no further knowledge of the development of the pharynx except that to be derived from general statements of the various positions it may assume in the regenerating pieces. More recently a paper by Bardeen on the physiology of *Planaria maculata* has taken up the formation of the pharynx in this species and given a somewhat detailed account of it.

In studying the origin and growth of the new pharynx, I wished to find out if there were any marked difference between its formation in the *new* tissue of the anterior piece and in the old tissue of the posterior piece, since the conditions for regeneration seemed so unlike in the two cases. At the same time I was interested in observing in the posterior pieces what might be the influence of the reproductive organs when present in the region where we should expect the pharynx to appear, and if, under these conditions, the already existing cavities and external opening can be made use of. In this connection I examined series of regenerating posterior pieces in which the cut had been made (1) just posterior to the pharynx, but at a time of year when the reproductive organs have disappeared; (2) anterior to these organs when they are fully developed; and (3) posterior to them.

The greater part of the material for my experiments was collected from ponds near Woods Hole during the months of June, July, and August, and was preserved for study in the following winter. Later in the year more planarians were found near Bryn Mawr, and were kept in aquaria until needed. Each worm was cut into three pieces, of which the middle, containing the pharynx, was rejected, and the anterior and posterior pieces were allowed to regenerate. The latter were killed at intervals of twelve hours for about eight days, at the end of which time those remaining had begun to feed. Where it seemed advisable, series were killed at closer intervals, but as a rule this was not necessary. The pieces remained in corrosive acetic for about ten minutes; after cutting they were stained on the slide in Delafield's hæmatoxylin, and then dipped for a few seconds into a strong solution of aniline orange. The latter differentiates the endoderm, muscle bands, yolk cells, and the lining of the reproductive system.

Soon after the worms are cut, the edges of the cut-end draw together and matter from the digestive tract and loose parenchyma tissue collect about the injured region. Within a few hours the surface opens out again, until only a slight depression marked by a dark line of pigment is noticeable. In the mean time parenchyma cells collect at the cut surface and form a narrow sheet across it, while cells from the margin of the uninjured epithelium push out and cover the new growth. This overgrowth of epithelium is comparatively rapid, as the cells in this region flatten out and cover individually a much larger area. By the end of the first day the tip has become rounded and the anterior end is covered with an epithelial layer; later, with the increase in their number, the flattened cells resume their columnar form.

The parenchyma of the normal planarian appears on examination as an ill-defined mass filling in the spaces between the organs. Its protoplasm stains very slightly, but the tissue is conspicuous owing to the many deeply colored nuclei that are present in it. These nuclei are occasionally seen in small groups, but they are usually scattered and separated from each other by the loose spongy tissue. Among the parenchyma cells are found other cells which are specialized in function,— large rhabdite cells, lying just below the ectoderm and showing the rhabdites buried in the protoplasm, and the mucous cells, staining an intense blue; also, along the digestive tract, a few large granular cells for which Bardeen has suggested a digestive function. In addition, I have noticed some large cells staining with aniline orange, which seem to contain some such material as yolk. Whether this is a fourth kind or merely the digestive cells I have not been able to decide. There are also present elongated connective-tissue cells.

Although the majority of the parenchyma cells show little protoplasm, scattered through the tissue of the normal uninjured animal there are a good many cells whose nuclei resemble those of the ordinary parenchyma, but which have gathered about them an irregular mass of granular protoplasm. As many of these are in various stages of division, while I have found no division among the cells with less protoplasm, it is probable that the accretion of protoplasm is the usual forerunner of division in the parenchyma cells, and that the number of the latter is constantly increasing even in normal planarians. While the number of these dividing cells differs individually, they are often very numerous and are confined to no one region of the body, except that they are more rare in the extreme tips. The dividing cells stand out very distinctly as they are larger and seldom have other cells near them.

After the animals are cut, the amount of division increases somewhat throughout the whole body, but very markedly near the injured surface. Cells also migrate down into this region and form there a thickening which rapidly enlarges. That migration takes place is indicated by the long trails of protoplasm that give to the cells a bipolar appearance with the axes directed towards the growing mass (Fig. 1), while in the normal planarian there is no such effect and the cells are more irregular and rounded in outline. After the thickening has reached a certain degree of concentration there is rarely any cell division in the proliferated mass, but mitotic figures can frequently be found in the region near by, where the cell mass is less dense. Cells also continue to migrate into the new part and add to the new growth. The number of cells in the parenchymatous tissue of the body itself is also usually much increased as the nuclei throughout continue dividing, and by the time the pharynx thickening appears, which is in the course of the third day, the tissue is filled with cells conspicuous on account of their greater amount of protoplasm.

I shall consider first the growth of the pharynx in the anterior piece and then that in the posterior piece. By the time the proliferation of cells in the regenerating posterior tip is quite marked, a slight shift in the direction taken by some of the cells denotes the beginning of the new pharynx. The cells now collect just on the line between the old and new tissue at the posterior end of the digestive tract, which has rounded off, and usually has begun to bud out side branches. As is true of the growing end, so here, when the thickening becomes marked, no division of cells can be found in it, but in the less dense tissue around it there are many dividing cells and apparently considerable cell migration towards the new growth. The collection of cells stains very deeply in comparison with the old tissue near by, due probably to the greater amount of protoplasm in this region.

The thickening for the pharynx grows until it is quite large, and then the split for the pharynx chamber begins. This opens up very rapidly as a narrow cavity running across the base of the thickening and up on the sides, being usually more advanced on the ventral than on the dorsal side. It is at first irregular in outline, but the cells along the edge soon flatten out into a thin lining epithelium. As development proceeds, cells are added at the anterior end of the new pharynx, while there is no division in the compact mass of cells already collected (Fig. 2). About the fourth day the lumen of the pharynx itself appears in the center of the proliferation, due to an elongation and pulling apart of cells which become larger and thin walled and form the lining of the pharynx. This cavity gradually enlarges posteriorly to open into the pharynx chamber, and anteriorly to unite with the wall of the digestive tract, which then breaks through to join with it. The new region has in the mean time steadily increased in length, and the branches of the intestine have pushed back into it, while the central digestive cavity has also somewhat enlarged. Shortly after the lining of the lumen has formed, the muscle bands of the pharynx differentiate. They arise from cells just between the two epithelial layers; they elongate and undergo some change in their constitution, since they stain with aniline orange very shortly after the lengthening process has begun. The change is first visible in the region where the pharynx is continuous with the body of the animal and extends gradually posteriorly. So far as I could determine, the circular muscles develop before the longitudinal, but if so, the difference in time is very slight. In the course of the fifth or sixth day the pharyngeal chamber opens to the exterior by an ectodermal invagination from the ventral surface, and shortly afterwards the animal begins to feed.

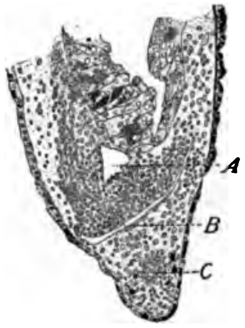


FIG. 2.

FIG. 1.

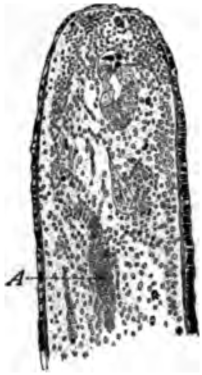


FIG. 3.

FIG. 1. — Large parenchyma cells migrating towards regenerating region.

FIG. 2. — Pharynx in anterior piece at close of fourth day. The lumen has just appeared. *A*, lumen; *B*, lateral digestive branch; *C*, pharynx chamber.

FIG. 3. — Pharynx in posterior piece at close of third day. The lateral digestive branches have met, and the pharynx thickening (*A*) is conspicuous. There are many dividing cells present.

The shape and size of the pharynx vary comparatively little after the inner lumen appears. At first, before the formation of this cavity, the shape is slightly pointed, but it soon becomes more regular and assumes the form of the normal pharynx, except for its length, which is only about once and a half or twice the breadth. The addition of cells at the anterior end does not continue after the structure is well organized, and the latter part of the time needed for the regeneration of this organ is occupied in changing the parenchyma cells into the different tissues. Up to the time when the muscles appear, the cells still remain crowded together and show by their deep color the presence of unusually dense protoplasm. After the muscles are well formed the tissue assumes a less dense appearance, and this change arises apparently at the same time in the cells throughout the new tissue. Deeply staining masses, as of mucous, can also be seen, so that the tissue soon resembles that of the normal worm.

The process of formation of a new pharynx in the old tissue of the posterior piece seems to be very similar, and equally simple during the nine months of the year when the reproductive organs are absent. The overgrowth of the cut surface and the increase in the number of dividing cells is just as was found in the anterior piece. The pharynx thickening, however, appears about twelve hours later than in the former case, *i.e.*, about the end of the third day, and is usually noticeable shortly after the two branches of the digestive tract have joined. But that the pharynx is formed with relation to the "axial gut," as Bardeen states, I am not prepared to say, as occasionally a case is found in which the branches have not met, although a thickening is present, and often in a slide showing a conspicuous gathering of cells the union between the intestines has barely taken place. The pharynx forms a short distance posterior to the cut, in the old tissue. It is first made visible by the shifting of the axes of some of the cells, which now direct themselves toward the new point of activity, and the thickening increases rapidly (Fig. 3). The cavities open up just as in the anterior piece, but the chamber is apt to be much longer and more irregular in outline than in the other case. Later the enlarged cavity rounds off so that the chamber resumes its normal size. The central cavity of the digestive tract enlarges in a posterior direction, and at the same time the pharynx thickening is added to by cells at the anterior end; thus, by the time the pharynx is ready to open up, the original space between it and the digestive tract is bridged over (Figs. 4, 5). The appearance and development of the lumen, muscle bands, and normal spongy tissue is identical with that in the anterior pieces, and the posterior piece is also ready to feed in seven days.

Summing up these results, it appears that the regeneration of the pharynx in the new and in the old tissue is much the same, (1) as to the origin and migration of the cells, (2) as to the formation of the two cavities (the chamber and the lumen of the pharynx), and (3) as to the differentiation of the cells into the normal tissues. They differ (1) in the length of time that elapses before the pharynx thickening shows itself (about twelve hours more being required for its appearance in the old tissue than in the new), (2) in the position of the developing pharynx with regard to the central digestive cavity, and (3) in the size of the pharyngeal chamber as shown by its early irregularities in the posterior piece. The last two differences are probably due to the disposition of the digestive branches in the two cases.

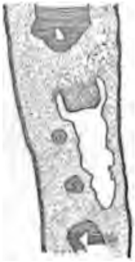


FIG. 4.

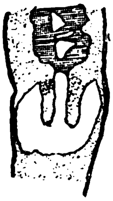


FIG. 5.

FIGS. 4, 5. — Stages in development of pharynx in posterior piece.

In the early part of the summer the reproductive organs of this species are fully developed, and regeneration under these conditions is especially interesting. The genital apparatus lies for the most part in the region just between the pharyngeal and genital pores, so that when an animal is cut just posterior to the pharynx these organs occupy the region where the new pharynx should develop. Under these conditions there is no sign of the growth for the new pharynx until towards the end of the fourth day, when a small collection of cells can be found in a ventral but not necessarily median position. Also this mass is usually at a normal distance from the fused intestine. Morgan (1900) has noted this delay in the formation of the pharynx when the worm is cut anterior to the genital pore at this time of year (July 2) and finds about twelve days instead of seven required for complete regeneration. His later experiments, begun July 31, gave different results, but this was probably due to the degeneration of the reproductive organs between these two dates, as he found that the time required for this set of pieces to become complete animals was only seven days.

This delay may be due to the scarcity of parenchyma cells in this region, which is almost completely filled by the tissue of the genital organs. When these organs are fully present the parenchyma cells collect as shown in Fig. 6, migrating principally from the anterior end into the region of parenchymatous tissue that lies just ventral to the penis. In this drawing are shown in the tissue along the edge of the genital organs certain irregular breaks that seem to appear at the beginning of the degeneration of these structures. The pharynx forms ventral to such cavities, and the split for the pharynx chamber is at first entirely independent of them (Figs. 6, 7). Later the wall between may break down, and thus greatly enlarge the chamber, but if so it is a secondary process; there is also a tendency towards degeneration in the thin line of tissue between the pharynx and reproductive chambers, as shown by the dotted line in Fig. 7. In no case, however, does the old genital pore become the new pharyngeal pore.

As the genital organs degenerate, which I have found takes place during the last two weeks in July and the beginning of August, the region in which the pharynx appears becomes quite irregular.. The only feature that seems at all constant is the relative distance between the cross-branch of the intestine and the pharynx thickening. That this is true may be seen by a study of its position with relation to that of the genital pore. The main genital duct opening to the exterior persists longer than any other part of these structures, but its presence seems to have little or no influence on the position of the new pharynx. The latter may appear far anterior to it, posterior, or, as in Fig. 8, it may assume an eccentric position and lie on the same level; in this case the duct and pore are shown by dotted lines, as the pharynx lies about eight sections to the side of the median line, *i.e.*, of the genital pore. Thus the new pharynx can be found in all possible positions, and the variations, such as lateral, ventral, and oblique, should be noted in connection with any hypothesis of the relation of the pharynx to the axial gut.

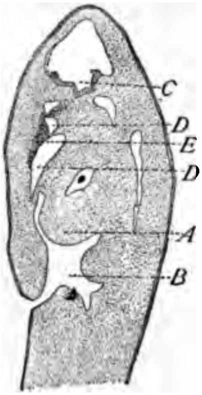


FIG. 6.

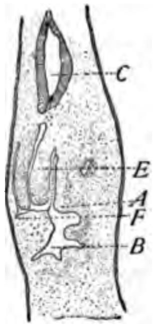


FIG. 7.

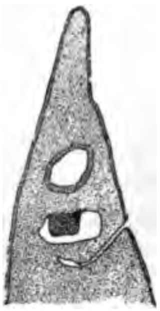


FIG. 8.

FIGS. 6, 7. — Development of pharynx during presence of reproductive organs. *A*, penis; *B*, genital chamber; *C*, digestive tract (in 6 the wall has broken down between it and the degenerating cavity); *D*, cavities due to degeneration of reproductive organs; *E*, pharynx; *F*, position of pharyngeal pore.

FIG. 8. — Position of regenerating pharynx with respect to genital duct and pore. The new organ appears to one side of the pore but on the same level. The dotted lines indicate direction of duct in following sections.

As regards the process of regeneration in the posterior pieces of worms cut behind the genital pore when the reproductive organs were believed to be fully developed, the process of formation seems identical with that which takes place during the absence of these organs. Consequently there is no need to describe what takes place.

I wish to express my indebtedness to Prof. T. H. Morgan, under whose direction this work has been done.

BIOLOGICAL LABORATORY, BRYN MAWR COLLEGE.

Sep.-Abdr. aus Roux' Archiv für Entwickelungsmechanik, Band XIV, Heft 3/4. 1902.

Further Experiments on the Regeneration of the Tail of Fishes

By

T. H. Morgan.

With 52 figures in text.

Eingegangen am 18. April 1902.

Some experiments that I made two years ago on the regeneration of the tail of certain teleostian fishes[1] gave evidence of the presence of a formative factor that regulated the rate of growth at different levels. The experiments showed that the rate of growth from an oblique surface is determined not by factors that would ordinarily be recognized as physiological ones, but by certain formative factors. For instance, when the tail of fundulus is cut off obliquely the most rapid growth of the new tail is in the region that lies nearest the base of the tail, and from this region of maximum growth the rate decreases regularly to the most distal part of the same surface, as shown here in Fig. 24. Expressed in one way it may be said that the rate of growth is greatest where most material is needed to complete the typical form of the tail. A better way of stating the result is that the rate of growth in different regions is regulated by the form assumed by the new part. A more convincing example of the same process is seen when a fish with a bilobed tail is used. If the tail is cut squarely off the new material, that appears over the cut-surface, begins to grow faster at two points corresponding to the upper and lower lobes of the tail. If the tail is cut off obliquely, two regions of more rapid growth again develop, one above and one below, but the latter increases faster than the former.

---

[1] Regeneration in Teleosts. Archiv f. Entwickelungsmech. X. 1900.

Its rate of growth corresponds to that of the lobes in a tail cut across[1]).

Since these experiments bear on some important problems of growth and of regeneration I wished to repeat them on a larger scale, and to extend them in other directions. This I have been able to do during the past summer while occupying the Bryn Mawr Table at the Marine Laboratory at Woods Holl. Later I continued the experiments on some gold-fish at Bryn Mawr.

I.

In my former work on fundulus I compared the rate of growth of the new tail, developing from an oblique surface, with the rate of growth of the new tails in other individuals in which the tail had been cut squarely off, and found that the maximum rate of growth, which takes place from the most proximal part of the oblique surface, corresponds to the maximum growth from the cross-cut surfaces — the rate of growth over the latter being about the same for all levels. Since some difference was founed in the rate of growth in different individuals it seemed desirable to compare the rates of growth from two cross-cut surfaces of the same tail. This can be done by cutting off the tail as shown in Fig. 1. The new growth takes place both from an outer (distal), and from an inner (proximal) cross-cut surface, as shown in Figs. 1, 2, 3. This experiment was carried out not only to determine the rates of growth at two different levels of the same tail, as stated above, but also to see if the growth over the outer cross-cut surface is held in check by the presence of a regenerating region at a more proximal level; the two cut-surfaces not being directly connected except by the longitudinal cut-edge connecting the inner ends of the two cross-cut surfaces[2]).

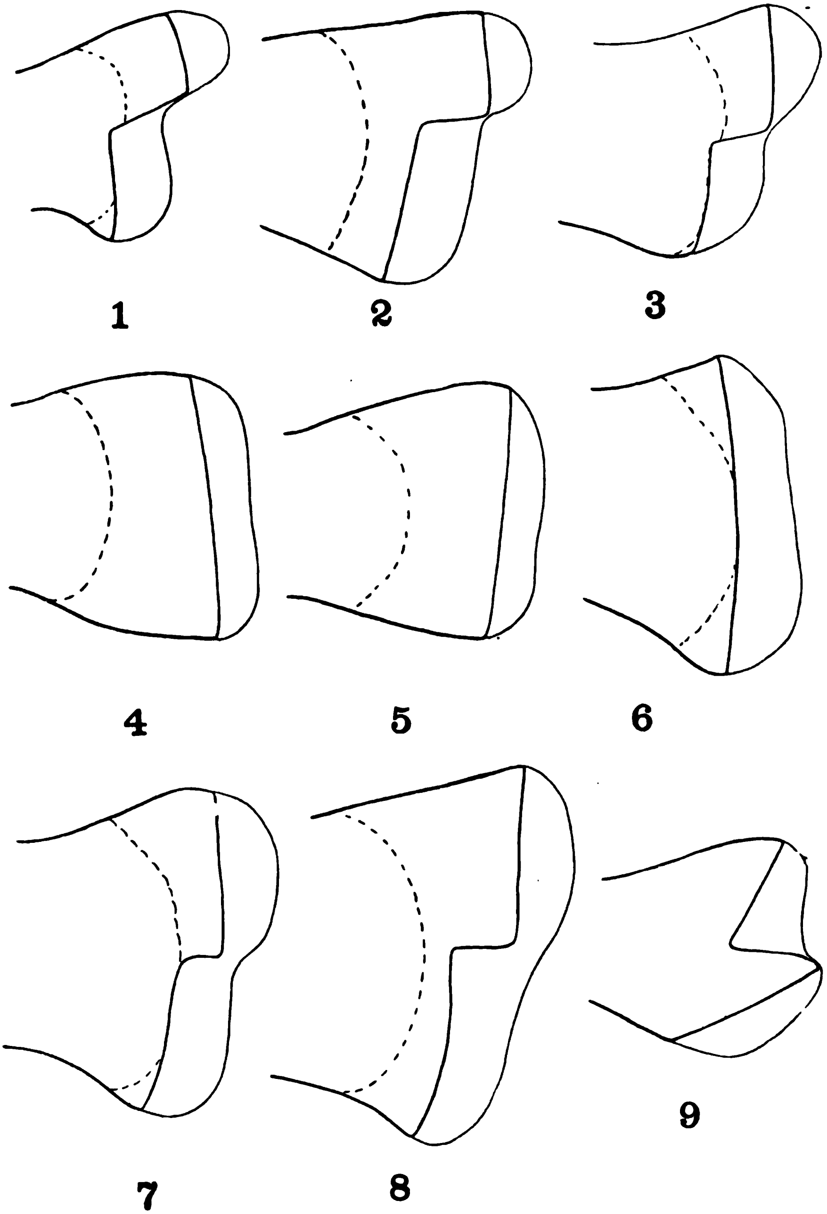
In Fig. 1 the rate of growth from the inner surface is somewhat greater than that over the outer surface. The two new parts are connected by a little new tissue near the middle region of the tail.

---

[1]) I have more recently repeated this experiment with **Menidia notata**, and have obtained the same result.

[2]) The figures in the present paper represent to better advantage the form of the tail and its relation to the new part, than do some of those in my former paper. This is owing to a better method of keeping the severed tail expanded. After killing the fish the tail was cut off and pinned out, fully expanded, upon a floor a paraffine in a dish of sea water. Formalin (4%) was then poured over the tail and it was later preserved in the same solution.

Figs. 1—9.



Each new part has a somewhat rounded form, i. e., the most rapid growth is near the middle. In Fig. 2 the same difference in the rate of growth over the two cut-surfaces is noticeable. In this case both cut-surfaces are nearer the outer end of the tail. In Fig. 3 the rate of growth is more nearly the same over the two cut-surfaces; both cut-surfaces are here nearer the base of the tail. These results may indicate either that the growth from the distal cut-surface is held in check on account of the new growth taking place at the inner cut-surface, or the growth may be always greater near the base regardless of the presence or absence of another growing region. Which of these interpretations is the correct one can only be determined by check experiments on other fish in which the whole tail is cut off at different levels as in my earlier experiments. In the series to which Figs. 1, 2, 3 belong there were only three fishes with tails cut squarly off, Figs. 4, 5 and 6. In Fig. 4 the tail had been cut off (slightly obliquely) near the outer end, in Fig. 5 near the middle, and in Fig. 6 near the base. The growth of the new tail is greater in the latter case.

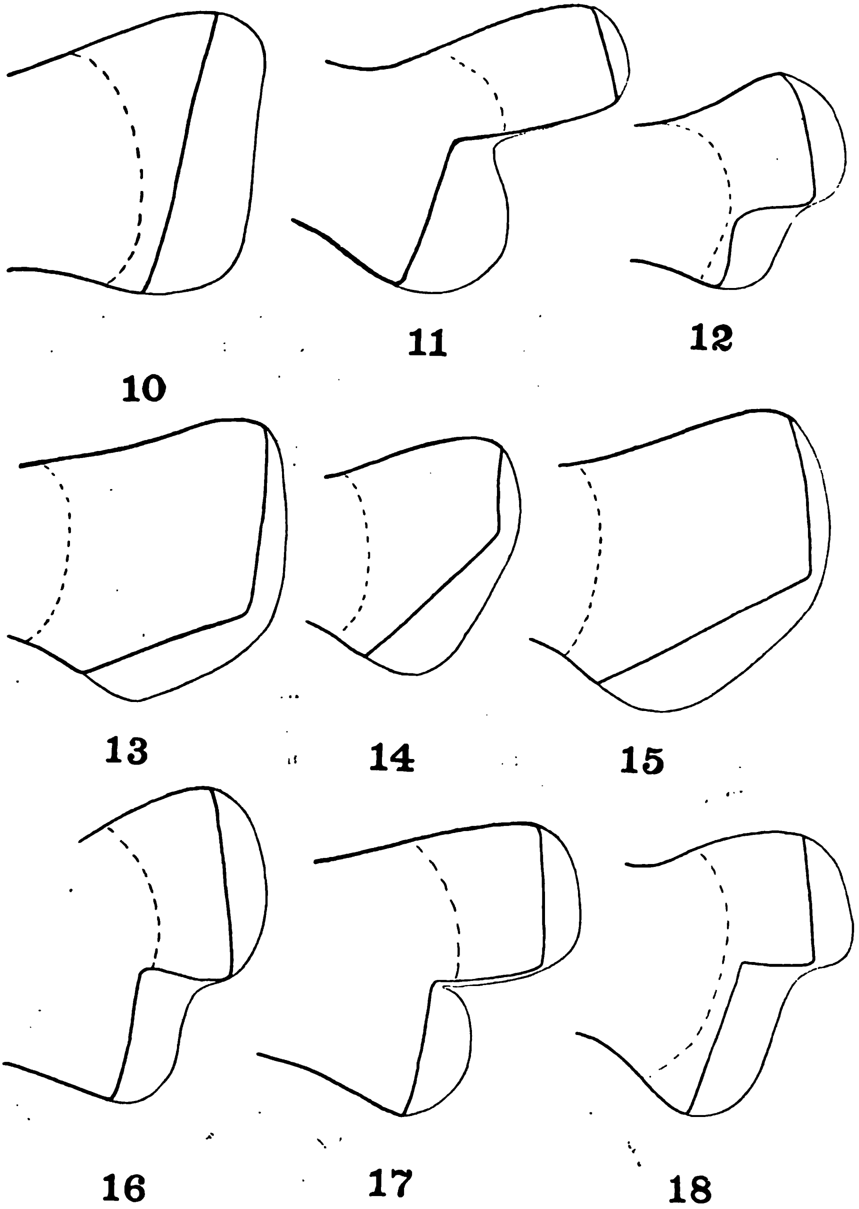
This result seems to show that the differences in the rates of growth at the two levels in Figs. 1, 2, 3 can be accounted for by more rapid growth taking place from a more basal region. In another fish the tail had been cut off somewhat obliquely, as shown in Fig. 10. Here the more rapid growth has been from the lower part, but the difference in amount of new tissue above and below is not sufficient in this case (because the cut is not very oblique) to enable as to judge whether it may not be accounted for by the same factors that determine the difference in Figs. 4 and 5.

In this same series two other tails were cut off as shown in Figs. 7 and 8. In these the outer and the inner cut-surfaces are nearer together than in Figs. 1, 2, 3. Both cross-cuts are near the base of the tail in Fig. 7, while in Fig. 8 they are further out. In both of these cases also the new part has grown somewhat faster from the inner cut-surface. Before commenting on the meaning of these results let us examine other series.

In another series represented in Figs. 12, 16, 17, 18, 19, the results are about the same as before, both when the inner and outer cross-cut surfaces are near together, as in Figs. 16 and 18, and when they are further apart as in Figs. 12, 17 and 19. There is moreover no noticeable difference in the rate of growth when one cut-surface is longer (from above downwards) than the other. In Fig. 12 and in Fig. 19 the growth is about the same over both the inner and the outer cross-cut surfaces.

From this evidence there does not seem to be any doubt that when two cross-cut surfaces are present on the same tail the new

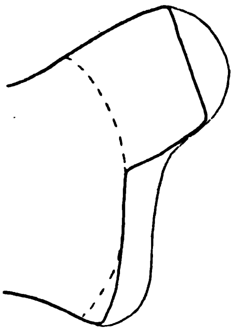
Figs. 10—18.



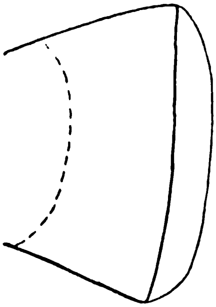
part generally grows somewhat faster from the inner of the two surfaces. Comparing this result with the growth when the whole tail is cut off squarely the conclusion seems highly probable that the difference in the rate of growth over the outer and inner cut-surface of the same tail is due to the region of the cut, and not to a regulative influence of one region on the other.

The question at once arises whether this same difference will account for the different rates of growth at different points of an oblique surface. The following experiments throw light on this question. In Figs. 20, 21 and 22 three tails are shown that had been cut off squarely, one near the base (Fig. 21), one near the middle (Fig. 20), and one near the outer end (Fig. 22). The new part in about the same in all three cases; rather more, in fact, in Fig. 22. In the same series two tails were cut off quite obliquely, Figs. 23 and 24. The growth at the lower part of each tail, is more than twice that at corresponding levels of the upper part of the same tail. This difference is much greater than that between the two tails cut off squarely at two levels (Figs. 21, 22), and also much greater than that between the outer and the inner new parts in Figs. 16, 17, 18 and 19 which belong to this same series. We are confirmed, therefore, in our earlier conclusion that the rate of growth from an oblique surface cannot be accounted for by the distance of the different regions from the base of the tail. We must also add, however, to our former conclusion the statement that a somewhat more rapid regeneration may take place from a cross-cut near the base of the tail than from a more distal level. This factor may also be present in the regeneration from an oblique surface, but in addition there is also present a regulative influence that holds in check the regeneration from the more distal parts of the new tail. In connection with the development of two new parts from two independent and discontinuous cross-cut surfaces, as shown in Figs. 1, 2, 3, 7, 8, 11, 12, 16, 17, 18, the question arises whether the new parts represent halves of a single tail or two new tails of smaller size. Attention has been called to the fact that each new part has a rounded form as a result of more rapid growth at the middle. This is strikingly shown in Fig. 17 and also in several of the other figures. In a sense the new part is obviously not a whole tail since it contains only half the characteristic number of rays, but this fact does not militate against the possibility of the new part being a whole structure. The fish in these lots were not kept long enough to show whether a new whole tail would develop from the outer and another from the inner cross-cut, or whether each would become the half of a single tail. All that was seen went to show that a single tail would develop, and

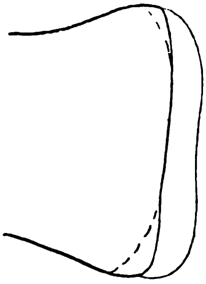
Figs. 19—27.



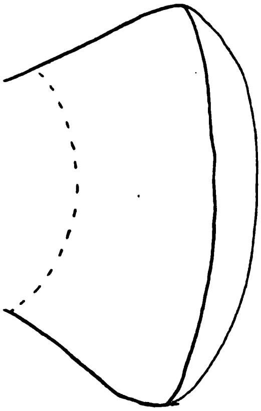
19



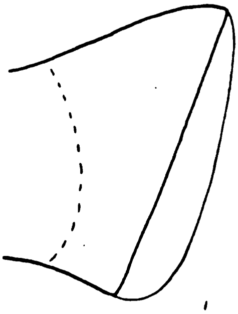
20



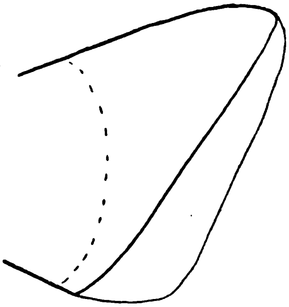
21



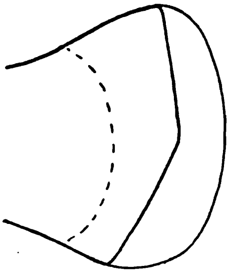
22



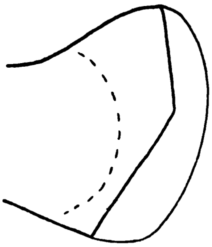
23



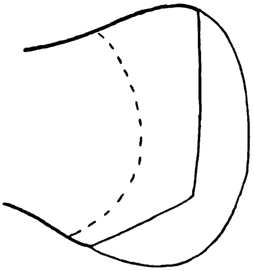
24



25



26



27

later observations have confirmed this conclusion beyond a doubt. In a case like that shown in Fig. 17 it might appear that a whole tail was forming from the lower half-surface. This is, however, not the case. Something has delayed the growth at the inner edge hence the rounded effect. In regard to the similar rounding observed at the upper and lower edges, the effect is only is part the result of the formative influence producing the rounded tail of this fish. It is also to be observed in fishes with a belobed tail, and seems to be due to the connection of the new part with the old, or rather to the same principle which determines that rounded outlines and not rectangular ones are produced — something connected, perhaps, with the semifluid nature of organic material.

II.

In several experiments the tail was cut off as shown in Figs. 13, 14, 15. The tail was first cut squarely off, and then by an oblique cut an additional part was removed from the lower (or upper) part of the tail. The purpose of the experiment was to determine if the rate of growth over the cross-cut surface is influenced by the growth over the oblique surface — the two surfaces being in this case continuous. The experiment differs from that in which the tail was cut off obliquely, not only in one surface being in this case squarely cut off, but also in the presence of a sharp angle around which the new part will be continuous. I also wished to determine if the relative lengths (dorso-ventrally) of the cross and oblique surfaces have an influence on the result, for, it seemed not improbable that a small oblique surface might not be able to hold in check the maximum possible growth over a much larger cross-cut surface.

In Fig. 13 the rate of growth of the new part from the oblique surface, especially from its lower edge, is much greater than the growth over the cross-surface. The length of the latter surface is more than half the height of the tail, but about equal in actual length to that of the oblique cut. In Fig. 15 the length of the cross-cut surface is about half the height of the tail, and less in actual length than the oblique surface. The rate of growth from the cross-surface is again less than that from the oblique surface; in fact only about one-fourth as much. In Fig. 14 the cross-surface is less than half the height of the tail, and the new growth from it much less than that from the oblique surface. These cases are especially instructive when compared with those represented in Figs. 16, 17, 18 of the same series. The latter figures show the rate of growth from two cross-cut surfaces, one near the base of the tail, and one near the outer end of the tail. The growth from the outer cross-cut of

Figs. 16, 17, 18 is much greater than that from the cross-cut part of Figs. 13, 14, 15.

Since the principal difference between the two experiments is the continuity of the new part in one case, and the lack of continuity in the other, it seems to follow that in this lies the difference in the rates of growth in the two cases. We conclude, therefore, that in Figs. 13, 14, 15 the growth over the cross-cut surface is held in check owing to its connection with the new part over the oblique surface. The presence of a sharp angle does not interfere with the action of one portion of the new tail on the other portion, and the results are nearly the same as when a continuous oblique surface is present.

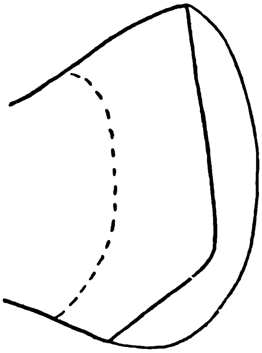
A similar result is shown in Fig. 25 from another series. In this case the cross-surface is about half the height of the tail. In Fig. 26 the cross-cut surface is less than half the height of the tail and the regeneration from its surface is less than that from the oblique surface. In Fig. 27, however, the cross-cut surface is much longer than the oblique surface, and although there is still some difference in the amount of growth from the two surfaces, the difference is less than in the preceding case, and can be accounted for, perhaps, by the more rapid growth from a more proximal region.

In three other cases belonging to another series, the tails were cut off as in the last experiment and the results are shown in Figs. 28, 29, 30. Owing to the shortness of the oblique surface the new part that develops from it seems to be unable to restrain the maximum rate of growth over the cross-cut surface. In another case in this series, shown in Fig. 31, the oblique surface is longer than the cross-cut surface, which is small, and we find that the amount of new growth from the latter is relatively less than in the preceding cases. In regard to these cases (Figs. 28, 29, 30) I should hesitate to state definitely that the results are entirely due to the greater length of the cross-cut surface in comparison to the oblique surface, although this conclusion seems the more probable one.

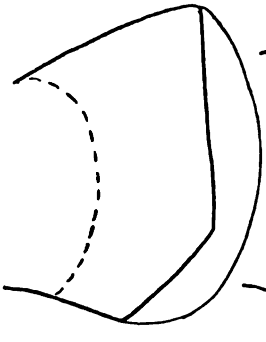
III.

It was not until late in the summer that I thought of the following experiment, and I regret that I could not carry it out more fully. A tail of fundulus was cut off as shown in Fig. 32, so that two oblique, half-surfaces are present (connected by a longitudinal cut-surface). The upper end, x, of the upper oblique surface is at the same distance from the base of the tail as the lower end, Y, of the

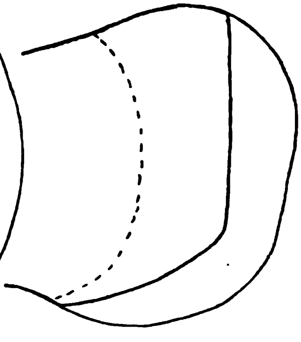
Figs. 28—34.



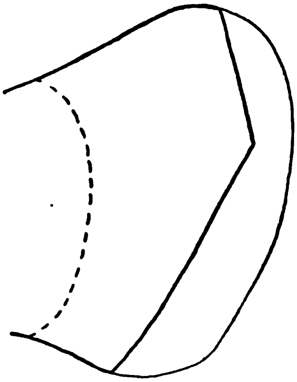
28



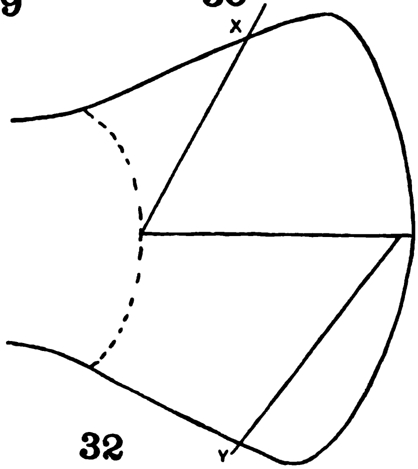
29



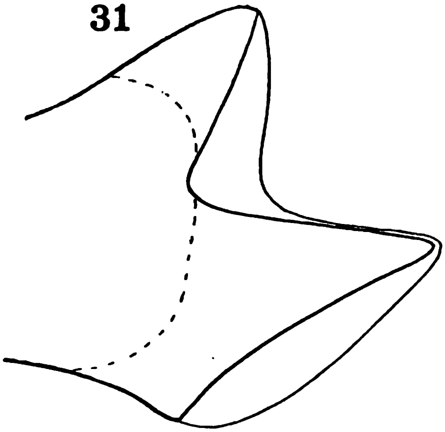
30



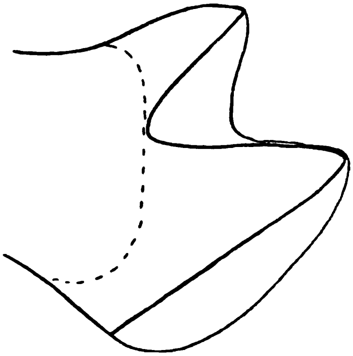
31



32



33



34

lower oblique surface. If the rate of growth is determined only by the level from which the new growth takes place we should expect to find the growth at x and at y to be the same, since they are both at the same distance from the base of the tail. The results in two cases are shown in Figs. 33 and 34. Here it is seen that the rate of growth at x is much less than at y. Another even more satisfactory case is represented in 9. Two other cases, not figured, gave similar results. This experiment shows clearly that other factors than the level at which the new part is forming also come into play in the formation of the new tail. That these factors are formative ones is shown not only by these new results on fundulus, and the earlier ones on fundulus, stenopus, macrella, and menticirrus, but by the experiments described in the following pages on the regeneration of the new tail in the common gold-fish.

IV.

A striking fact observed in the regeneration of tails cut off as shown in Figs. 11 and 17 is that there is no regeneration in a dorsoventral direction from the cut-edge that connects the two cross-surfaces. The results is obviously connected with the absence of the cut-ends of fin-rays. If a tail is cut off obliquely the new rays do turn somewhat downwards (or upwards as the case may be) but when the cut is made in a horizontal direction, passing between two of the rays no regeneration, except that involving the healing over of the cut-surface, takes place.

That this result is really connected with the absence of the cut-ends of the fin-rays, and is not a phenomenon of direction, is shown by cutting across the dorsal or the anal fin. In the former case regeneration takes place vertically upwards, in the direction of the fin-rays, and in the latter case vertically downwards, as I have shown for this same fish. Again of the pectoral or pelvic fin is cut off cross-wise, regeneration takes place from the cut-end, and, therefore, in a lateral direction. In other words the fish regenerates in the three dimensions of space, and the direction that is followed is determined in each case by the structural relations of the old part.

If we look further into this process we get a certain insight into the meaning of the phenomenon. New fin-rays are formed only from the ends of preëxisting ones. When the tail — or any other fin — is cut lengthwise between two rays, none of the rays are exposed along this edge and the edge simply heals over, or at most produces material corresponding to that between two rays. If, however, a fin-ray should grow out from the inner cross-surface into the new material along the edge, new material may then be, Fig. 11, added along the ventral surface. The results may be formulated by stating that the direction of regeneration is determined by the presence or absence of existing structures — the fin-rays — that serve as points of departure for the new structures. Were it possible to split a ray itself lengthwise there can not be much doubt that it would complete itself along the cut-surface and having done so there would be no further exposure of the material of the rays, hence no further regeneration. In this we find an explanation of the absence of regeneration along a longitudinal cut-surface.

This conclusion has a bearing on the results of WENDELSTADT[1]) in which one of the bones of the fore arm of the salamander was removed, being disarticulated at both ends. As could certainly have been foreseen the remaining bone of the fore arm did not regenerate laterally to reproduce the missing bone. Only a misconception of the principles involved in regeneration could have lead the author to expect the replacement of the lost bone under these conditions.

V.

The tail of the gold-fish (Carassius auriantiacus) has a bilobed form and regenerates readily, as BROUSSONET first showed in 1786. Owing to the bilobed form of the tail of this fish a number of problems can be more readily worked out than on fundulus.

The tail of one fish was cut off obliquely as indicated in Fig. 35. The new part began soon to grow[2]) faster at the upper and the lower regions than in the middle, Fig. 35, and the lower lobe grew faster than the upper one, Figs. 35, 36. This result agrees in all respects with my former one on stenopus which has also a bilobed tail.

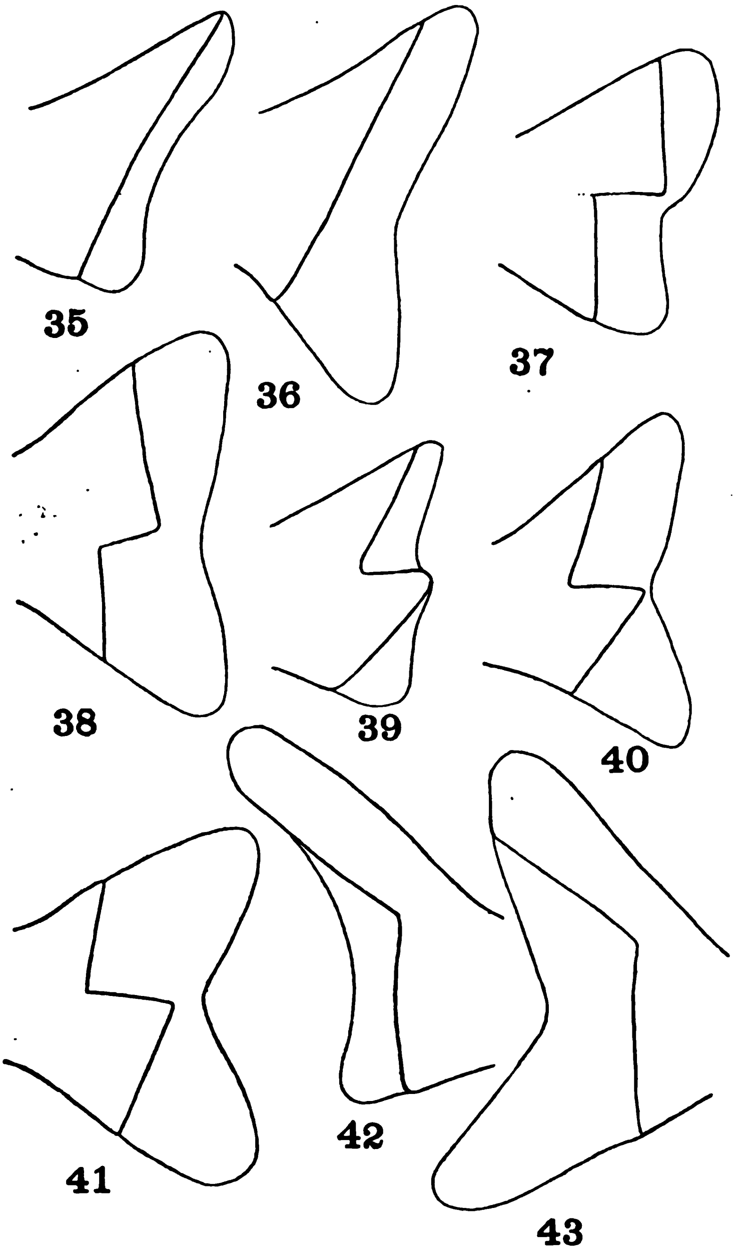
The tail of another fish was cut off as shown in Fig. 37. Two half cross-cuts were present at different levels. The object of the experiment was to see whether from each cut-surface a half tail (with one lobe) or a whole tail with two lobes would develop. The new

---

[1]) Über Knochenregeneration. Archiv f. mikr. Anat. LVII. 1901.

[2]) The figures of the gold-fish were made in all cases from the living fish, hence the same tail is represented at consecutive periods of growth. The fish was wrapped in wet filter paper with its tail projecting. It was then laid on the stage of a dissecting microscope and the tail drawn with the camera. The fish was then returned to the water.

Figs. 35—43.



part that appeared on each cross-cut surface soon began to grow faster at the upper, and at the lower side producing a single lobe of the tail. Later, as shown in Fig. 38, when the two parts of the tail have extended beyond the middle of the old part the bilobed form of the new tail is clearly seen.

It is important to note that only after the more proximal half has grown up to the level of the outer cut-edge does any considerable growth take place from this region. The experiment shows clearly that from each half a half-tail develops, although at first the two halves are not directly connected except along the horizontal edge connecting the two cross-cut surfaces.

The tails of two other fishes were cut off as shown in Fig. 39. Two oblique cut-surfaces are present connected along the middle line by the horizontal cut-edge. The formation of the new tail is shown in Figs. 39, 40. In both cases a half-tail with its lobe is formed on each oblique surface. If the rates of growth in the middle region at the upper end of the lower oblique surface and the lower end of the upper oblique surface are compared, it will be seen while from the one very little new material is formed, from the other a great deal is produced. This difference in the two regions in question is probably not due to their relative distance from the base of the tail, but rather to a formative factor holding in check the growth from the outer region. After the new part from the more proximal surface has extended outwards to the end of the old part the region that has been held in check up to this time begins to elongate, extending backwards until it reaches its definite position, Fig. 41.

It will be seen in this last experiment that in one case at least the upper edge of the upper half is at the same level as the lower edge of the other half. Since, however, in this fish the new lobes are produced from these two regions the experiment cannot be used to illustrate the same points as in the case of fundulus.

The tail of another fish was cut off as shown in Fig. 42. The tail was split lengthwise between two fin-rays along a line a little above the angle of the tail. Then by a cross-cut the larger portion was cut squarely off. In this case the exposed cross-cut surface is somewhat more than half the height of the tail. The object of the experiment was to determine how much of the new tail develops from this cut-surface. As shown in Fig. 42 more than a half-tail was produced from the cut-surface. It finally grow out completing the part of the old tail that remained, Fig. 43. A similar result is shown in

Fig. 44, in which the notch is even more clearly seen. In this case the tip of the remaining old part was also cut off and has regenerated.

In another case, Fig. 45, a tail was split lengthwise below the fork of the tail and then by a cross-cut the smaller, lower portion was remowed. The operation was carried out in order to see if less than a half-tail would develop from the small cross-cut. The result is shown in Figs. 45, 46. The new part is less than a half-tail and represents little more than the lobe of the tail.

In the foregoing experiments the absence of regeneration along the horizontal cut-surface is very noticeable.

In two other cases, after an operation similar to that of Fig. 37, the longitudinal cut-surface was extended into the basal part of the tail along the dotted line. By severing in this way the connection between the lower part (with its cross-cut surface) and the upper part I hoped to find out whether a whole tail or only, as before, a half tail would appear on the lower cross-surface. I found that the separated parts grew together, and although I separated them three or four times at intervals of several days the longitudinal cut-edges persisted in fusing together. A half tail developed from each cut-surface as in the other cases in which the upper and lower parts of the tail had not been separated.

These experiments on the gold-fish show convincingly that half-structures and incomplete structures develop from half cross-cut surfaces or from surfaces more or less than half, and it seems probable from the last experiment that this would occur even if the halves or parts were completely separated from each other. The explanation of this lies largely no doubt in the influence of the old part along the cut-edge on the new part. It seems to me improbable, in the light of these results, that the presence of the old part beyond the cut-surface, i. e. the old part completing the rest of the tail, has any important influence on the results and probably no influence at all.

Another experiment which is in many respects complementary to one of the last, consists in cutting off the tail as indicated in Fig. 47. The tail is first cut squarly off, then a longitudinal cut is made below the middle of the tail and the lower piece cut squarely off at the base. The outer, longer cross-cut surface is more than half the height of the tail. Will it produce a corresponding part of the tail with a part only of the lower lobe or a whole tail? In the simplest case, like that shown in Figs. 47, 48, 49, the new part that develops on the outer cut-surface is more than half, but not a whole tail since part that appeared on each cross-cut surface soon began to grow faster at the upper, and at the lower side producing a single lobe of the tail. Later, as shown in Fig. 38, when the two parts of the tail have extended beyond the middle of the old part the bilobed form of the new tail is clearly seen.

It is important to note that only after the more proximal half has grown up to the level of the outer cut-edge does any considerable growth take place from this region. The experiment shows clearly that from each half a half-tail develops, although at first the two halves are not directly connected except along the horizontal edge connecting the two cross-cut surfaces.

The tails of two other fishes were cut off as shown in Fig. 39. Two oblique cut-surfaces are present connected along the middle line by the horizontal cut-edge. The formation of the new tail is shown in Figs. 39, 40. In both cases a half-tail with its lobe is formed on each oblique surface. If the rates of growth in the middle region at the upper end of the lower oblique surface and the lower end of the upper oblique surface are compared, it will be seen while from the one very little new material is formed, from the other a great deal is produced. This difference in the two regions in question is probably not due to their relative distance from the base of the tail, but rather to a formative factor holding in check the growth from the outer region. After the new part from the more proximal surface has extended outwards to the end of the old part the region that has been held in check up to this time begins to elongate, extending backwards until it reaches its definite position, Fig. 41.

It will be seen in this last experiment that in one case at least the upper edge of the upper half is at the same level as the lower edge of the other half. Since, however, in this fish the new lobes are produced from these two regions the experiment cannot be used to illustrate the same points as in the case of fundulus.

The tail of another fish was cut off as shown in Fig. 42. The tail was split lengthwise between two fin-rays along a line a little above the angle of the tail. Then by a cross-cut the larger portion was cut squarely off. In this case the exposed cross-cut surface is somewhat more than half the height of the tail. The object of the experiment was to determine how much of the new tail develops from this cut-surface. As shown in Fig. 42 more than a half-tail was produced from the cut-surface. It finally grow out completing the part of the old tail that remained, Fig. 43. A similar result is shown in

Fig. 44, in which the notch is even more clearly seen. In this case the tip of the remaining old part was also cut off and has regenerated.

In another case, Fig. 45, a tail was split lengthwise below the fork of the tail and then by a cross-cut the smaller, lower portion was removed. The operation was carried out in order to see if less than a half-tail would develop from the small cross-cut. The result is shown in Figs. 45, 46. The new part is less than a half-tail and represents little more than the lobe of the tail.

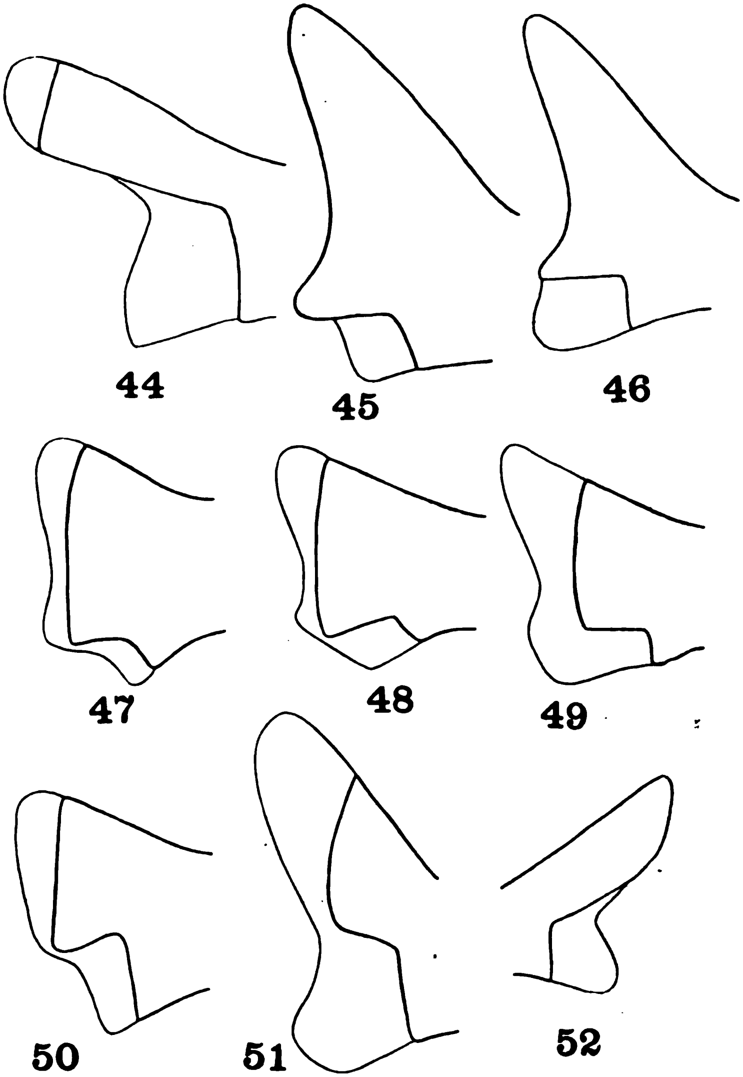
In the foregoing experiments the absence of regeneration along the horizontal cut-surface is very noticeable.

In two other cases, after an operation similar to that of Fig. 37, the longitudinal cut-surface was extended into the basal part of the tail along the dotted line. By severing in this way the connection between the lower part (with its cross-cut surface) and the upper part I hoped to find out whether a whole tail or only, as before, a half tail would appear on the lower cross-surface. I found that the separated parts grew together, and although I separated them three or four times at intervals of several days the longitudinal cut-edges persisted in fusing together. A half tail developed from each cut-surface as in the other cases in which the upper and lower parts of the tail had not been separated.

These experiments on the gold-fish show convincingly that half-structures and incomplete structures develop from half cross-cut surfaces or from surfaces more or less than half, and it seems probable from the last experiment that this would occur even if the halves or parts were completely separated from each other. The explanation of this lies largely no doubt in the influence of the old part along the cut-edge on the new part. It seems to me improbable, in the light of these results, that the presence of the old part beyond the cut-surface, i. e. the old part completing the rest of the tail, has any important influence on the results and probably no influence at all.

Another experiment which is in many respects complementary to one of the last, consists in cutting off the tail as indicated in Fig. 47. The tail is first cut squarly off, then a longitudinal cut is made below the middle of the tail and the lower piece cut squarely off at the base. The outer, longer cross-cut surface is more than half the height of the tail. Will it produce a corresponding part of the tail with a part only of the lower lobe or a whole tail? In the simplest case, like that shown in Figs. 47, 48, 49, the new part that develops on the outer cut-surface is more than half, but not a whole tail since the notch is not in the middle but nearer the lower end. What we must regard as the rest of the lower lobe is developing on the smaller proximal cut-surface.

Figs. 44—52.



44 45 46 47 48 49 50 51 52

It can be shown that there is in this experiment at least one other factor that has an influence on the lower end of the new part developing from the outer cut-surface. The connection of this part with the new tissue along the longitudinal (horizontal) cut-edge inhibits the growth to a greater or less extent of the incomplete lobe of this region. The influence may be of a formative nature in the sense that it is the result of the connection with the new tissue forming on the inner cut-surface, or the influence may be of a different, or physical nature, and simply be the outcome of the nearness of this region to a longitudinal edge along which new tissue is developing but only to a limited extend. I have at present no means of deciding between these alternative. One thing, however, seems fairly established, viz., that unless the longitudinal cut is made some little distance below the level of the notch of the tail the part of the lobe does not appear. This result is shown in Figs. 50, 51. In this case[1]) the part of the lobe is prevented from developing until the new part from the inner, shorter cross-cut surface has reached the level of this region. As it grows beyond it the missing part is restored.

These experiments on the gold-fish leave little doubt that the development, from a surface less than the whole, is incomplete, i. e. a whole structure never develops from a part of the old structure.

VI.

A number of experiments were made on the tail of a fresh water sun-fish. The tail regenerates as readily as do those of all the other fishes that I have examined. The result of one experiment is shown in Fig. 52. In this case the tail had been cut off in the same way as that of the gold-fish of Fig. 42. The cross-cut surface is longer than half the height of the tail, and has produced more than a half structure. The fin-rays that run to the notch of the new tail come from the cut-ends of the old middle rays. The rays in the new part above and below this level come from the cut-ends of the old ones in corresponding portions. The more rapid development along the horizontal cut-edge is due to the extension into this region of the new rays from the cross-cut surface, and not to new material from the longitudinal cut-edge.

VII.

During the winter '00—'01 I carried out a few experiments on the tails of gold-fish to determine the relation between the black pigment that is present in some fish in the old and in the new tail. Taking a fish with a narrow black band at the most posterior edge,

---

[1]) There were 12 fin-rays in the upper part and 8 in the lower.

I cut the tail off some distance proximal to this band. The new part arose, therefore, from a non-pigmented part of the old tail. Nevertheless the new tail developed a new pigment band after it had grown to a certain length. In another case the tail was cut off through the black band itself, and here, as might have been anticipated, the black pigment appeared in the new part. I found also that if a tail that has no black pigment in it is cut off the new tail does not develop black pigment.

Results similar to these were also observed in several of the experiments on gold-fish described in the preceding pages. The result shows that the pigmentation of the tail is an individual character, which is restored during regeneration, even when the entire pigmented region has been cut off. The new material arises in this case from a part of the tail that is not pigmented and which does not itself subsequently produce pigment. In one or two cases I observed that the pigmentation of the new part differed in certain details from that of the old, but as a rule the same sort of pigmentation — whether bands or spots — comes back.

I had hoped to be able to repeat this experiment on fish in which definite specific markings, such as an eye-like speck is present, but as yet I have not obtained the necessary material. The bearing of the experiment is obvious, since in general it is found that structural features are only regenerated when some substratum remains composed of material similar to that in the organ to be regenerated. When, for instance, the leg of a salamander is cut off the foot develops from the proximal cut surface provided the latter contains the different elements — bones, muscles, blood-vessels, nerves, skin, etc. — of which the foot also is composed. If, however, the entire leg is cut out — disarticulated — a new limb does not regenerate. In case of pigment markings it may appear at first sight that the removal of the entire pigmented part — as the band at the end of the tail — would correspond to the removal of the entire limb of the amphibian, but this may not be the case, since the presence of pigment-cells throughout the tail may supply the necessary elements for the formation of a new pigmented region.

VIII.

The earliest mention of regeneration in fishes that I have been able to discover is a paper of Broussonet[1]) written in 1786. In this he states that he examined the power of regeneration in many kinds of fishes, and at different times of year, and found that the fins »reproduce themselves« by slow degrees. He found that regeneration takes place most quickly in young fishes, and in some species of fish better than in others; also that the fins of the gold-fish — Poissons dorés de la Chine — would regenerate, and that after three months the new rays could be distinguished, and after eight months the »right fin«, which had been cut off in one case had become as large as the left one. The ventral fin also regenerated as well as the caudal fin after oblique or transverse cuts.

---

[1]) Observations sur la régénération de quelques parties du corps des Poissons. Hist. de l'Acad. Roy. des Sciences. 1786.

Broussonet makes the following statement that shows that he accepted Bonnet's view of a connection between liability to injury and the power to regenerate: »Y'ai remarqué que les nageoires se réparoient d'ordinaire plus ou moins promptement suivant qu'elles étoient plus ou moins utiles à l'animal«. Spallanzani he says made similar observations on earthworms. As evidence in favor of his statement Broussonet points out that the caudal fin which is the most useful of all the fins regenerates faster than the ventral or the pectoral, and that the lateral regenerate better than do those of the back. In the latter the rays appear only after eight months.

In order that the fin may regenerate it is necessary to leave a part of the »osselets«. If these are destroyed new fins are not produced as is shown when the dorsal fin together with a portion of the back is cut off. A suture is formed at the wound, but not a new fin. Broussonet records that he had seen quite a large fish, which had lost not only the tail but also a part of the body lying behind the anus, remain alive for several years. He also states that if the tail of a fish is split lengthwise between the rays the two parts unite subsequently.

Mazza¹) published in 1890 a short account of the regeneration of the caudal fin in certain fish. He does not seem to have known of Broussonet's results. In fact, these seem to have been lost sight of almost altogether in recent times. I did not know of them when I wrote my first paper, and in the literature given by Barfurth no mention is made of Broussonet, or of his work. Nussbaum and Sidoriak²) also seem to have overlooked this early paper.

---

¹) Sulla ringenerazione della pinna caudale in alcuni Pesci. Atti Soc. Ligust. Sc. Nat. I. 1890.

²) Archiv f. Entwickelungsmech. X. 1890.

Mazza having found a fish with an abnormal fin thought that this might have been due to incomplete regeneration. Therefore he set to work to find out if the tail of fishes possess the power of regeneration. He cut off a part of the tail of the gold-fish (Carassius auratus) and found that regeneration took place. A short account of the histological condition of the new tail is given.

A recent paper[1] by Jósef Nussbaum and Szymon Sidoriak gives an account of the regeneration of the tissues of young brook trout. The fish were operated on a day after hatching. The tail-end was cut squarely off. In some individuals only the tail-fin was cut off, in others a larger part of the posterior end, the cut passing posterior to the anal opening, and in others the cut was made in front of the anus. The closing of the wounded surface by ectoderm, and the later multiplication of cells in the chorda, muscles, connective tissue and nerve cord, is described in detail. In the experiment in which the cut had been made in front of the anus a new posterior opening is established as well as a new opening for the urethra. The young fish died as a result of the growth of parasitic moulds before the replacement of the lost part had taken place.

Summary.

1) When two half cross-cuts are made on the same tail of Fundulus, as in Figs. 1, 2, 3, 4, 5, the new part grows somewhat faster from the more proximal half. This difference appears not to be due to any regulative influence of the two new parts on each other. The difference in the rate of growth of the two half-surfaces is much less than that between the outer and inner parts of a oblique surface, Fig. 23. In the latter case these is a regulative (formative) influence which holds in check the growth of the new tissue in the outer parts of the oblique surface.

2) When the tail is cut off both by a cross-cut and an oblique cut, as shown in Figs. 13, 14, 29, we must distinguish three different cases, a), b), c). a) When cut off, as in Fig. 13, so that the cross-cut and the oblique-cut are of equal length, the new part that arises from the oblique surface has a regulative effect on the rate of growth of the new part arising from the cross-cut-surface; b) when cut off, as in Fig. 14, so that the oblique surface is longer than the cross-

---

[1] Archiv f. Entwickelungsmech. X. 1900.

cut-surface a similar regulative influence is observed; c) but when the oblique cut is shorter than the cross-cut-surface, as in Fig. 29, the new part arising from the former does not appear to be able to hold in check the growth from the cross-cut surface.

3) When the end of the tail of Fundulus is cut off by two oblique cuts, as shown in Fig. 32, so that the upper cut-edge of the upper oblique surface is at the same level as the lower cut-edge of the lower oblique surface, the new part grows out as shown in Figs. 33 and 34. The rate of growth from the upper cut-edge of the upper oblique surface is much less than that from the lower cut-edge of the lower oblique surface, although these two edges are the same distance from the base of the tail. The result is unquestionably due to a regulative influence of the new parts developing on the two oblique surfaces. If the experiment is reversed in respect to dorsal and ventral surfaces the results are the same.

4) The rounding of the new half-part that sometimes occurs, as in Figs. 11 and 17 is not to be interpreted as the development of a whole tail from a half cut-surface, but is the result of some check on the growth along the longitudinal cut-surface which unites the two cross-cuts.

5) Regeneration of new parts always takes place lengthwise in the direction of the fin rays, and practically no new part at all is formed along the cut-edge of a longitudinal cut-surface, Fig. 11.

6) The regeneration of the bilobed tail of the gold-fish, Carassius aurantiacus, is similar to that of Stenopus chrysops which has a tail of the same form. The rate of growth from an oblique cut is shown in Figs. 35, 36. The more rapid rate of growth of the lower lobe is evident. The method of regeneration after two cross-cuts, and after two oblique cuts, is shown in Figs. 37—41.

7) When somewhat more than the half of the tail is cut off by a cross-cut, as in Fig. 43, the new part grows more slowly in the structural middle region of the new tail, so that the bilobed form is ultimately restored. If a piece less than half the height of the tail is cut off, as in Fig. 45, the lobe only of the tail grows out, Fig. 46.

8) If two cross-cuts are made, as in Fig. 47, so that one of them is longer than half the height of the tail, the new part that developes on this surface grows more slowly in the structural middle of the tail, so that an upper lobe is formed, and a part of the lower lobe. The rest of the lower lobe develops on the shorter cross-cut surface. There is a regulative influence (or else an in-

fluence of some other sort) that partially keeps in check the growth of the part of the lower lobe developing on the longer cross-cut-surface.

9) The results show that an incomplete structure, and not a whole one develops from a cross-surface less than the entire height of the tail.

10) If the tail of a gold-fish, having a black band (or splotches) at the end, is cut off so that the entire band is removed a new black band appears on the new tail. The presence of a part of the old band is not necessary in order that a new one shall be formed.

Zusammenfassung.

1) Macht man (vgl. Fig. 1—3) zwei Querschnitte halb durch den Schwanz von Fundulus, so entwickelt sich der neugebildete Theil etwas rascher auf der mehr proximalen Hälfte. Diese Verschiedenheit scheint nicht auf einen gegenseitigen regulirenden Einfluss der beiden neuen Theile auf einander zu beruhen. Die Wachsthumsverschiedenheit zwischen den beiden Schnitthälften ist viel geringer, als die zwischen den inneren und äußeren Theilen eines Schrägschnittes (Fig. 23). Im letzteren Falle besteht ein regulirender (formativer Einfluss, welcher das Wachsthum an den äußeren Theilen der schrägen Schnittfläche hemmt.

2) Wird der Schwanz (wie in Fig. 13, 14, 29), gleichzeitig durch einen queren und einen schrägen Schnitt abgetrennt, so müssen wir drei verschiedene Fälle (a, b, c) unterscheiden: a. Wird der Trennungsschnitt (wie in Fig. 13) so geführt, dass Quer- und Schrägschnitt gleiche Länge haben, so hat die sich von der schrägen Schnittfläche aus erhebende Neubildung einen regulirenden Einfluss auf das Maß des Wachsthums der Neubildung an der Querschnittfläche; b. Wird (wie in Fig. 14) durch die Schnittführung die Schrägschnittfläche länger gemacht als die Querschnittfläche, so sieht man einen ähnlichen regulirenden Einfluss. c. Ist jedoch (wie in Fig. 29), der Schrägschnitt kürzer als der Querschnitt, so scheint die von ersterem sich erhebende Neubildung nicht im Stande zu sein, den vom Querschnitt ausgehenden Wachsthumsprocess in Schranken zu halten.

3) Wird das Schwanzende von Fundulus (wie in Fig. 32) durch zwei Schrägschnitte abgetrennt, so dass die obere Ecke des oberen Schrägschnittes mit der unteren des unteren sich in gleichem Niveau befindet, so erfolgt das Wachsthum der Neubildung, wie in Fig. 33 und 34. Der Wachsthumsbetrag an der Oberecke ist dabei viel geringer, als der an der Unterecke, obwohl beide von der Basis des Schwanzes gleich weit entfernt sind. Es liegt dies unzweifelhaft an einem regulirenden gegenseitigen Einfluss der Neubildungen an den beiden Schrägschnittflächen. Kehrt man den Versuch in Bezug auf Rücken- und Bauchfläche um, so bleibt das Resultat dasselbe.

4) Die Abrundung der neuen Hälfte, die bisweilen eintritt (Fig. 11 und 17), darf nicht als die Bildung eines ganzen Schwanzes seitens einer halben Schnittfläche gedeutet werden, sondern als der Ausdruck eines Wachsthumshindernisses im Verlaufe des Längsschnittes, welcher die beiden Querschnitte verbindet.

5) Die Bildung der neuen Theile findet stets in Längsrichtung, in der der Flossenstrahlen statt und in Wirklichkeit entsteht nicht die geringste Neubildung entlang dem Wundrande einer Längsschnittfläche.

6) Die Regeneration des zweitheiligen Schwanzes vom Goldfisch (Carassius aurantiacus) ist der des Schwanzes von Stenopus chrysops ähnlich, welcher einen Schwanz von gleicher Form besitzt. Den Wachsthumsbetrag von einem Schrägschnitt aus zeigen Fig. 35 und 36. Der schnellere Wachsthumsfortschritt des unteren Lappens ist deutlich. Die Art der Regeneration nach zwei Quer- bezw. zwei Schrägschnitten zeigen Fig. 37—41.

7) Wenn Einiges mehr als die Hälfte des Schwanzes entfernt durch einen Querschnitt wird (wie in Fig. 43), so wächst die Neubildung in der Strukturmitte des neuen Schwanzes langsamer, so dass die zweilappige Schwanzform schließlich wieder hergestellt wird. Wird ein Stück von weniger als der halben Höhe des Schwanzes abgeschnitten, wie in Fig. 45, so wächst nur der Schwanzlappen aus.

8) Werden zwei Querschnitte angelegt (wie in Fig. 47), so dass der eine durch mehr als die halbe Höhe des Schwanzes reicht, so wächst die an dieser Schnittfläche sich entwickelnde Neubildung langsamer in der Strukturmitte des Schwanzes, so dass der obere und ein Theil des unteren Lappens hier entsteht. Der Rest des Unterlappens entsteht an der Fläche des anderen kürzeren Querschnittes. Auch hier tritt ein regulirender (oder sonst wie gearteter) Einfluss auf, der das Wachsthum des dem Unterlappen entsprechenden Theiles bei der Entwickelung an der längeren Querschnittfläche beschränkt.

9) Die Versuchsergebnisse zeigen, dass nur eine unvollständige, keine ganze Bildung sich von einer Querschnittfläche entwickelt, die weniger als die ganze Schwanzhöhe beträgt.

10) Schneidet man einem Goldfisch mit am Ende schwarz gebändertem Schwanz diesen so ab, dass das ganze Band verloren geht, so erscheint auf dem neuen Schwanze ein neues schwarzes Band. Das Übrigbleiben eines Theiles des alten Bandes ist für die Regeneration eines neuen nicht erforderlich.

Experimental Studies of the Internal Factors of Regeneration in the Earthworm.

By

T. H. Morgan.

With Plates XXIX and XXX.

Eingegangen am 18. April 1902.

Sep.-Abdr. aus Roux' Archiv für Entwickelungsmechanik, Band XIV, Heft 3/4. 1902.

My study of the regeneration of the earthworm, Allolobophora foetida, began in the spring of 1892. The results of this work, extending through the winter of 1893—94 were published in the Quarterly Journal of Microscopical Science, XXXVII. 1895.

During the winters of 1895—96 and '96—'97 I carried out some further experiments which were described in Roux's Archiv. V. 1897. Since then I have carried on, during each collegiate year, additional experiments extending over the four years between 1898—1902. Two of the principal results, that I have obtained, have been briefly described, the one in the Anatomischer Anzeiger. XV. 1899, and the other in my book on »Regeneration« 1901. page 53. The following pages give a complete account of the experiments of the last four years.

Removal of a Piece of the Mid-ventral Wall Containing the Nervous System.

This experiment was first carried out on Oct. 13 '99. My object was to see what would happen if the nervous system, which other results had indicated acts as a center for the formation of the new part, is removed from the anterior cross-cut surface. Will a new head be formed, I asked myself, and if so will it have a nervous system not connected at first with the old one? And further, will a new head develop from the mid-ventral region where the old nervous system ends?

The worms were anaesthetized with ether, and the anterior five or six segments were cut squarely off. Then the blade of a pair of small oculist's scissors was pushed into the body cavity, just on one side of the mid-ventral line, and a cut made. A similar cut was then made on the other side of the mid-ventral line. The small mid-ventral piece of the ventral wall, with its part of the nerve-cord, was cut off by a cross-cut[1]), Fig. 33. In the first experiments the longitudinal edges of the cut were not drawn together after the operation, but I found that they often flared open, and the anterior end of the worm sloughed off. Sometimes, however, the sides united and good results were obtained.

In all later operations one or two (if the cut was long) stitches were made along the open part, and the walls drawn together by tying the threads. Under these conditions the anterior part less often died.

Not infrequently new tissue appears along the mid-ventral line, and a head slowly develops at or near the anterior cross-cut surface. This most often happens where the piece of the ventral wall that is removed extends only over two or three segments[2]). When the piece is longer the results are often different, and most of the following cases are the outcome of this kind of operation.

Case I. Fig. 1. In this case the ventral wound has closed and no regeneration has taken place from the anterior end of the worm. At the posterior end of the healed, ventral surface, where the cut-end of the old nerve cord lies, a papilla-like outgrowth is present (better seen in side view). Sections show that the new part is a head having a small brain and connectives that unite ventrally into a cord which is continuous with the anterior end of the old nerve-cord of the worm. A mouth opening leads into a tube which enlarges as it extends into the old part. From this enlargement a narrow tube runs forward in the anterior end of the old part to become continuous there with the old digestive tract, of which, no doubt, it was a part. The old digestive tract continues beyond this for a short distance to end blindly at the anterior end of the old part.

---

[1]) The appearance of the worm after the operation is also given in Fig. 26 in my book on »Regeneration«.

[2]) In such cases the ventral nerve-cord is also regenerated and the new head at the anterior end arises in connection with the anterior end of the new cord.

Case 2. Fig. 2. In this worm also there is an outgrowth on the ventral side near the anterior end. It is obviously a head. It lies at the proximal end of the healed over, ventral surface. Sections show that the outgrowth arises near the anterior end of the old nerve-cord, from one side of which a nerve-cord runs into the ventral part of the new head, Fig. 21. This cord divides on each side of the oesophagus, and the two branches unite dorsally with a small brain. The mouth leads into a short buccal tube and this into a well marked pharynx with a dorsal muscular wall (in which the brain is imedded). Behind this a short oesophagus opens into the side of the old one. This occurs a little posterior to the level of this section (Fig. 21). The old oesophagus runs forward to the anterior end of the old part where it ends blindly. The old nerve-cord extends only slightly in front of the level at which the nerve to the new part is given off. The extension is due to new material which spreads out irregularly. The cord ends, however, almost immediately after giving off the lateral cord to the new part.

Case 3. Fig. 3. In the mid-ventral line there is a broad area of new tissue from the middle of which a rounded protuberance arises. There is also at the anterior end a small projection of new material. Sections show that the latter is only the new tissue covering the anterior end of the old digestive tract. There is nothing to indicate that it is a head. The more posterior projection from the ventral nerface is more conspicuous. The old nerve-cord ends just behind it, but I cannot find any evidence that it extends into the projection. The old digestive tract runs past this region to end blindly near the anterior projection.

Case 4. Fig. 4. This case is different from the preceding cases in as much as there is a new head at the anterior end, composed of 3 or 4 new segments. In addition there is a smaller outgrowth further back at the posterior end of the new ventral tissue. It lies somewhat to one side. Sections show that the operation had failed of its main purpose since the piece removed from the ventral region had been cut off somewhat obliquely, as indicated in the diagram, Fig. 35. In consequence a small piece of the old nervous system was left at the anterior end, and in connection with this the new anterior head has developed. The posterior outgrowth has arisen in connection with the other anterior cut-end of the old nerve cord.

In the anterior head a ventral cord and brain is present as well as a pharynx connecting with the end of the old oesophagus. The posterior outgrowth has no distal opening. It contains a relatively large nerve cord which rounds up at its anterior end as though forming a brain, but, in the absence of a digestive tract, the brain, the connectives and the ventral cord are all one mass. The body wall of this outgrowth is made up of ectoderm, circular and longitudinal muscles. There is a central cavity traversed by fibrous strands. A few setae and seta-sacs are present in the wall. The nervous system joins the anterior end of the main ventral cord. The old digestive tract — the oesophagus — runs by the region of this outgrowth without sending a branch into the new part.

Case 5. Fig. 5. The anterior end has bent over somewhat. The only outgrowth is the one near the posterior end of the new tissue on the ventral side. The sections show that a mouth is present in the new part and that there is a pharynx which connects with the old gizzard on the ventral side. The old digestive tract extends forward into the anterior part, and there terminates blindly. The old nerve cord does not extend further forward than the new head. The head contains a brain, connectives and ventral cord which connects with the anterior end of the old cord.

Case 6. Fig. 6. This worm is particularly interesting since the new part, that arises at the posterior end of the new area, is quite a long distance from the anterior end. Sections show that the new projection is a head with mouth, pharynx, brain, connectives. The anterior end of the old nerve-cord grows out into the new part to form its ventral side. Behind the new pharynx there is a short oesophagus which joins one side of the old gizzard. The old digestive tract extends forward to the anterior end where it terminates blindly.

Case 7. Fig. 7. The anterior cut-end has formed a new head, somewhat on the ventral side. In addition there is another new outgrowth at the anterior end more on the dorsal side which sections show, Fig. 23, is also a head. The larger and more ventral head has a brain, connectives, and ventral cord connecting with the anterior end of the old nerve-cord. It has also a mouth, pharynx and oesophagus connecting with the old digestive tract.

The more dorsal outgrowth, Fig. 23, has also a mouth leading into a buccal tube, and this into a pharynx which connects with an oesophagus and the latter opens into a dorsal branch of the old oesophagus. In this dorsal head there is a brain with connectives uniting below the buccal tube with a short ventral cord, or piece of a cord.

This is quite unconnected with the old cord or with any part of the nervous system in the ventral head.

How can we explain the presence of two new heads with mouths, etc., each connecting with the old digestive tract? The most probable explanation is that a piece of the old ventral cord was left accidentally near the anterior cut-end, and around this as a center the dorsal head has developed. The other, the ventral head, has developed in connection with the anterior end of the old ventral cord.

Case 8. Fig. 8. In this worm the new outgrowth is not far from the anterior end. Sections show a new head has developed with a mouth, opening into a dilated oesophagus (or pharynx) which connects with the anterior end of the old digestive tract. The latter extends somewhat beyond the new head, but its anterior ends bends over to become continuous with the new digestive tract. A brain, etc., is present in the new head.

Case 9. Fig. 9. In this worm a peculiar outgrowth is present at the anterior end. It arises somewhat on the ventral side, but the absence of a new ventral area indicates that the old anterior end has degenerated down to the level of the present anterior end. Sections show that a large mouth lies below the new outgrowth. The mouth leads into a new buccal tube and pharynx, and the latter opens into the old oesophagus. A part of the old oesophagus runs forward as a branch dorsal to the new pharynx. A brain lies above the new pharynx just anterior to the region where the dorsal part of the old oesophagus ends.

Case 10. Fig. 10. The relation of the parts is clearly seen in the figure. From the posterior end of the new ventral area an outgrowth having four rings is present. It has a prostomial-like tip at the end. No new part has arisen from the anterior end of the old worm. Sections show, Fig. 25, that the old nerve-cord extends into the new outgrowth, filling up almost all its hollow interior. At the anterior end it turns up sharply and a more dorsal projection appears to represent a brain. The ectoderm at the anterior end dips in for a short distance to form a stomodoeum. The inturning takes place beneath the prostomial-like tip. The old digestive tract runs past the region from which the new structure arises, and ends blindly in the new tissue at the anterior end of the old part. This new tissue, as shown in Fig. 10, projects slightly outwards. It shows, however, no indications of forming another new head.

Case 11. Fig. 11. In this case a small, but long outgrowth arises from the posterior end of the new ventral area. It is composed of rings, and has a terminal unsegmented piece. In addition there is a projection of new tissue at the anterior end. In surface view this showed faint indications of having a constricting dividing it into two rings(?) Sections show that this anterior projection in only a collection of new tissue around the anterior end of the old digestive tract. It is not so prominent as appears in the surface view. It does not appear to be a new head. The other, the posterior outgrowth with its five or six segments does not arise immediately from the anterior end of the old nerve-cord, but the latter extends forward for a short distance as a new cord, and then turns sharply outwards to enter the new part. It lies, within the body-wall and fills up a relatively large part of the central cavity, Fig. 22. At its anterior end it appears to enlarge, although on account of the obliquity of the sections in this region, it is difficult to be certain on this point. The body wall is composed of the characteristic layers. Setae seem to be absent. There is no stomodoeal ingrowth at the free end.

Case 12. Fig. 12. An outgrowth of four or five rings with a prostomial-like tip grows out of the ventral side near, but not at the anterior end of the worm. The worm was cut into sections, so that those of the new part were cross-sections. A wide mouth opens beneath the prostomium, this leads into a tube flattened from above downwards, above which lies a well developed brain, Fig. 24. This connects by its connectives around the digestive tract with a ventral cord. Further back a well-formed pharynx is present with a thick dorsal muscular wall. Where the new part arises from the worm, both the ventral nerve-cord and the pharynx extend over into the new part, the one to become continuous with the anterior end of the old cord; the other with the old digestive tract, whose anterior end is continuous with the digestive tract of the new part.

Case 13. Fig. 13. This worm is similar in many ways to the last. A ventral outgrowth of three rings is present near the anterior end. Sections show that a mouth is present at the end of the new part which leads to a buccal tube, and this to a new pharynx which lies partly in the new, partly in the body of the old worm. The nervous system hardly extends at all into the new part, since it bifurcates at its end and sends a pair of commissures around the pharynx to a large brain imbedded in the muscles of the upper side of the pharynx.

Case 14. Fig. 14. There is a long anterior old part of at least six segments, without a new head at its anterior end. About the level of the seventh segment a ventral outgrowth is seen with a terminal opening. Sections show that this is a head with mouth, buccal tube, brain, commissures and ventral cord. The buccal cavity connects with a small tube that runs forward almost to the anterior end to connect there with the old digestive tract. There is also present in this anterior end a part of the old digestive tract ending blindly except for the connection with the tube just mentioned.

Case 15. Fig. 15. In this case also there is a long anterior part of the old worm of about six segments which is closed and has produced no new structures at its anterior end. The new outgrowth arises from the seventh segment and from the top of a bulging part of the ventral wall. Sections show that the piece of the ventral wall had been removed somewhat obliquely, so that, as in Fig. 35, a part of the mid-ventral wall with its piece of the old nerve cord was left at the anterior end. Despite this a new head did not arise from this end, but it must be remembered that even when a worm is simply cut across a new head may sometimes fail to develop. The old digestive tract also extends into this anterior part. The new head arises in connection with the anterior end of the proximal, or main part of the old nerve-cord. The head contains a large ventral cord ending abruptly in front. There is no digestive tract in the new part. A slight ingrowth of cells at the free end may represent a stomodoeum. Setae are present in two of the proximal segments. Blood vessels extend from the old into the new part. There are no nephridia present.

In a few cases a different sort of operation was performed, Fig. 34. After cutting off five or six of the anterior segments a split was made along the ventral line (a little to one side) and from the posterior end of this cut a piece of the ventral-wall was cut out with its piece of the ventral cord, Fig. 34. The object of this experiment was to discover whether a head would arise at the anterior cut-surface, and another at the posterior end of the injured ventral region. At both points an anterior cut-end of the nerve cord is present. In most cases a head formed only at the anterior cut-surface, but in several cases a head appeared also at the posterior level.

Case 16. Fig. 16. A new head of three segments grew out at the anterior end. Another outgrowth with a central opening appeared five segments back in the new ventral tissue. Sections show that the anterior head has all the characteristic head-structures. The anterior piece of the old ventral cord comes to an end posteriorly where the piece of the ventral wall had been cut out. At the posterior end of the latter region and, therefore, at the anterior end of the posterior part of the old nerve-cord the second outgrowth arises. It represents a head also, with ventral cord, commissures, brain, and with mouth, buccal tube and pharynx just within the old part. The old digestive tract has, therefore, two communications with the exterior — one in each head. There are also two brains etc., present.

Case 17. Fig. 17. In this case a new anterior end appeared, but there was no new outgrowth from the injured ventral region. In healing one the two sides of the ventral area united very irregularly, as shown in the figure.

Case 18. Fig. 18. There is in this worm no new part at the anterior end except a small rounded projection in the middle of the new part. At the posterior end of the new ventral area an outgrowth having three or four segments is present. It has a curiously shaped end. Sections show that although a piece of the old cord is left in the anterior part a new head has not developed from its anterior end. The digestive tract is also present in this anterior part.

The posterior outgrowth arises at the anterior cut-end of the old cord. It seems to be a head, but there is no brain or brain-like enlargement of the end of the cord and no stomodoeum. The digestive tract does not extend into this outgrowth.

In the earlier experiments a number of worms were operated upon in the same way as in cases 1—15, but at the posterior end. None of them gave satisfactory results, because the segments from which the ventral piece had been removed constricted off and regeneration began further forward. It was, in fact, only after several unsuccessful attempts of this sort that I turned my attention to the anterior end where the body wall is more resistant.

In a few other cases the small piece of the ventral wall, containing the nerve cord on its inner surface, was not cut off. Operations of this sort were made both at the anterior and at the posterior end, but no results of interest were obtained, because the small piece either sloughed off, or more often reunited with the ventral region from which it had come. In none of these experiments did I sew together the sides of the cuts. Had I done this the results might have been different. If the experiment succeeded it would be interesting to see if a new head (or tail) would regenerate from the end of the small piece. I shall in the future repeat this experiment on a larger scale.

Summary and General Conclusions.

The influence of the nervous system. The results recorded in the preceding pages show that unless the cut-end of the nervous system is present at the cut-end of the worm a new head does not regenerate. This result, taken in connection with those from oblique cuts, shows unmistakably that the cut-end of the nervous system is the center around which the new part regenerates. Each of the different kinds of tissue can no doubt regenerate its like without the presence of the nervous system, but that the new tissues shall become organized into a new whole structure the presence of the nervous system is requisite. This conclusion may have nothing to do with the function of the nervous system as a center of nervous influences, but may, as other experiments which I am having carried on seem to show, depend simply on its structural importance, as one of a group of organs that go to make up a new part.

An equally important conclusion is that a new part composed of a head and of a number of segments may be formed without there being a digestive tract in the part. Under these conditions while the stomodoeal invagination may be formed, yet it does not develop very far, and does not produce the missing parts of the digestive tract. Although the new part lacks a digestive tract, the body wall is perfectly formed and contains the same layers as does the wall of the normal worm. The layers are continuous with those of the old part. Setae may develop, but they are few and not regularly arranged. Nephridia and septa appear to be absent. The irregular bands or threads in the central cavity may represent septal structures.

The absence of the nephridia is difficult to explain and their absence in some cases can hardly be accounted for by the new segments being these immediatly behind the new head, since a sufficient number of segments is sometimes present to lead one to expect the formation of nephridia in them. I shall hope to gain some insight into this result by carrying out similar operations on the posterior end of anterior pieces of worms. The absence of septa may account for the result.

In the absence of a digestive tract it is not surprising that commissures and brain are not well differentiated at the anterior end of the new head, for there are no structures present around which the commissures could form. The enlargement and dorsal extension of the nervous system that was observed in a few cases appear to represent an attempt to produce these structures.

Whether the presence or the absence of a digestive tract in the new part depends upon whether or not the old digestive tract was injured on its ventral side can only be determined by further experiments. That it may be injured is certain; that it is sometimes not injured, or at least not cut, I can also state to be true.

The relatively large size of the nerve-cord in the anenteric forms is very conspicuous. The ventral side of the new part seems to be determined by that side into which the nerve-cord extends and this is the region nearest the cut-end of the old cord.

Blood vessels although present are not in most cases conspicuously developed.

Those cases in which, as in Fig. 34, a piece of the old nerve-cord is present anterior to the region from which the ventral piece of the body wall is removed show that it is not necessary in order to produce a new head to have a continuous nerve-cord, but a piece of one will suffice. The anomalous case, described as case 7, can easily be explained as due to the presence of a small piece of the ventral cord left accidentally at the anterior end. Also the exceptional case, described as case 4, is undoubtedly due to this same factor. The sections show, in fact, the piece of the old ventral cord that was left. Theoretically as many new heads would develop in a worm as there are anterior exposures of the nerve-cord.

It is interesting to find that a new nerve-cord does not develop along the line of union of the lateral cut-edges provided a new head arises at the posterior end of this new tissue. We must certainly ascribe the absence of the ventral cord to the absence of a cut-end of the old cord in this region. Without a cut-end a new nerve-cord does not develop.

In conclusion one may summarize these results by stating that the presence of a cut-end of the nerve-cord is necessary at a cut-surface in order that a new head may develop. On the other hand the presence of the digestive tract is not necessary for the formation of a new head and segmented part. The absence of the digestive tract seems, however, to inhibit the complete formation of a stomodoeum, and likewise of a brain and commissures as structures quite distinct from the anterior end of the ventral cord.

The Bearing of the Preceding Experiments on Some Forms of Abnormal Annelids.

Andrews[1]) has given a very complete account of the external and internal structure of a number of oligochaete and polychaete annelids. Two of these forms were Al. foetida. In these an additional tail was found at one side near the posterior end of the worm. Dissections and sections of one worm show that all the characteristic organs are present in the side tail (as well as in the main one) and that the longitudinal organs are continuous with those of the trunk. In the second individual there is no digestive tract present in the outgrowth which is made up of eight rings and a terminal piece (without an anal invagination). The body wall has the characteristic organs (muscles, dorsal and ventral setae, ventral nerve-cord, and nephridia). A peculiar strand of muscles forms »a slender cord running the whole length of the body-cavity dorsal to the nerve-cord«. »It may, perhaps, be regarded as a representation of the musculature of the digestive tract.« The proximal end of the nerve-cord is continuous with that of the main trunk.

In a marine annelid Podarke obscura, Andrews found a number of similar abnormalities. The relation of the nervous system in some of the individuals calls for special comment. In several cases the nervous system is continuous into one tail-end but not into the other. If we assume, as Andrews also thinks probable, that one at least of the two tails is a regeneration-product, we must distinguish between the cases in which the nerve-cord in the new part is continuous with that of the trunk and those in which the nerve-cord in the old tail is not continuous etc. It is not always possible to make out which of the two tails is the new one, and in some cases both may be new.

My results on the earthworm can, I believe, he applied to the interpretation of these different conditions, although it must, of course, remain an open question whether in Podarke the nervous system is necessary for regeneration to occur. But if it prove true that this is the case as it is in the earthworm, the following hypotheses may be offered.

1) If a worm is wounded on its ventral surface so that the nerve-cord is cut in two, a new tail may grow out from the place of

---

[1]) Some Abnormal Annelids. Quart. Journ. Micr. Sci. Vol. 36. 1894. See also Friend, H., Nature. Febr. 1893.

injury in the ventral cord, and if the old tail remains a two tailed worm results. The old nerve-cord in the old tail may fail to unite with the cord from which it has been disconnected.

2) The injury may be of such a kind that a piece of the nerve-cord is broken off and separated from the rest. It may act as a center for the formation of a supernumerary tail. In such a case the new nervous system may appear to be unconnected with the old, but a careful examination should show a piece of the old cord at its proximal end.

3) The end of the worm may be so broken off that the nerve-cord is separated into two or more pieces and from each as a center a new nerve-cord may arise.

I am able to give here a description of another case of a worm having two tails. This specimen was found amongst a lot of normal worms and was given to me by Miss KATHARINE FOOT. In the region of the 41st—43rd segments a second tail (of 21 segments) comes off to one side, as shown in Fig. 30. At the base three of its segments (pigmented in the figure) contain parts of the old worm. I could not definitely determine whether the larger (of 47 segments) of the two tails, which is more nearly continuous with the old part, is a new tail or part of the original worm. Sections show that all the longitudinal organs are continuous into both tails. The digestive tract bifurcates, as do also the ventral nerve-cord, and the dorsal and ventral blood vessels. Septa, setae, nephridia, etc. are present in both tails. The conditions are, therefore, similar to those of the two abnormal individuals of Al. foetida, described by ANDREWS. The bifurcation is probably the result of an injury which involved splitting or crushing the ventral nerve-cord.

False Union of Metameres.

In 1892 I made a study of the abnormal rings that often occur in Annelids¹). CORI at the same time and independently gave an account of the same conditions. I found that some of the simpler and more characteristic abnormal unions may arise in egg-embryos and I offered as a suggestion to account for the split rings, compound rings, and spirals the hypothesis that the mesodermic somites sometimes

---

¹) A study of Metamerism. Quart. Journ. Micr. Sci. Vol. 37. 1892.

made union above or below with an incongruous segment of the other side. I also found by direct experiment that many of the most irregular forms of abnormalities arose from regeneration, being due both to false unions between the new and the old parts, as well as to irregularities in the new parts. My more recent results, especially those in which regeneration takes place from an oblique surface, fully confirm my earlier conclusions. The experiment of removing a piece of the ventral wall has also given rise to some most bizarre forms of union. Two cases may be here cited. In Fig. 20 it will be seen that a union has been formed between the middle of a segment and the second segment behind it. Again in the worm shown in Fig. 17 a most irregular union between the right and left sides has taken place across the mid-ventral line. Many other cases might be given, but these suffice to show how very extreme unions may occur, and under natural conditions it is not at all improbable that pieces might be torn or broken from the sides of the body, and in consequence equally irregular union of the old segments might take place.

Regeneration of Short Pieces From the Posterior End.

In one of my earlier experiments I had found that short pieces (of 10—12 segments) from the posterior end of the worm often failed to regenerate from the posterior cut-surface. It was not evident from the experiment whether the lack of regenerative power was due to the smallness of the piece, or to a real lack of power to regenerate in this region of the worm. In order to clear up this point I cut into pieces the posterior half of twenty-five worms, keeping pieces from the same region together. Each piece contained from 10 to 15 segments. I wished to determine, first, whether the power of regeneration from the posterior end of these pieces is different at different levels — the pieces being all of about the same size; second, whether fewer posterior segments would be produced by pieces that lay nearer to the posterior end of the old worm; third, whether the anterior ends of these pieces regenerated, and if so, whether a head or a tail was produced.

The experiment began Oct. 1. When examined after two weeks (Oct. 15) the following records were made. The roman numerals, I—V, give the position of the pieces; the first, I, being the piece next to the posterior end.

I, (Six pieces alive), no regeneration at the posterior ends[1]). II, (12 pieces), two showed the beginning of a new posterior part. III, (8 pieces), two showed a small new tip, and the others no new part, or only doubtfully a new beginning. IV, (8 pieces), five had new small posterior ends, three had very small ends, smaller than the preceding. V, (6 pieces), five had new ends; one had not regenerated.

On Oct. 30 the following results were recorded: I, (5 pieces), no regeneration had taken place at either end of any of the pieces. II, (10 pieces), in one of these a new short tail was present. III, (7 pieces), four pieces had a new short tail and one of the pieces had a new anterior part (head or tail?). IV, (6 pieces), all regenerating at the posterior end. The longest of the new tails was longer than the longest in III. V, (5 pieces), four regenerating; the new parts being about as long as those in IV. One piece was not regenerating.

On Nov. 13 the following results were recorded: I, (3 pieces), no new parts. II, (5 pieces), two of these had new posterior ends. III, (6 pieces), three of which were regenerating short posterior ends which were longer than those in the last. IV, (7 pieces), all regenerating at posterior end; one had 20 new segments. In these the new end was much longer than in the three cases in III. Two of the pieces in this lot had new anterior ends (whether head or tail uncertain). V, (4 pieces), all regenerating new tails as in last lot. Two pieces had new anterior ends (tails?).

On Dec. 4 the following results were recorded: I, (4 pieces containing 12, 13, 14 14 old segments respectively), none had new posterior ends. II, (5 pieces containing 9, 9, 11, 13, 15 old segments), two had short new tails (one of five segments), one was dying, and two had no new tails. III, (5 pieces containing 11, 11, 12, 13, 14 old segments), all had new posterior ends (one of these of seven, and another of eleven new segments). IV, (6 pieces containing 12, 12, 12, 14, 14 old segments), all had relatively long posterior ends of several to many new segments. Three of these pieces had anterior ends, one of which was composed of fifteen segments and was undoubtedly a tail, as was also another. V, (2 pieces containing 11 and 15 old segments), both having rather long new posterior ends and one with a new anterior end (head or tail?).

On Jan. 9. I, (one piece), no new segments. II, (3 pieces), with

---

[1]) When nothing is said to the contrary the pieces were not regenerating at the anterior end.

a small short posterior end one of four segments and this piece had a short anterior end of a few segments (head or tail?). III, (4 pieces), with new posterior ends of 12, 8, 4, 2 (or 3) new segments. One piece had a short anterior new part. IV, (4 pieces), with new longer posterior ends of 22, 20, 15 new segments, and another of many new segments. V, (2 pieces), one with a new posterior end of fifteen new segments and another of several new segments. One piece had a little new material at the anterior end.

Jan. 22. I, (one piece) no new material. II, (3 pieces) one with four new posterior segments (and also with four new segments at the anterior end), another with three new posterior segments and another with three and a half new segments. III, (3 pieces) with four, six and three new posterior segments. The latter seemed to have a little new material at the anterior end. IV, (4 pieces) with 16, 20, 20, 10 (or more) new posterior segments. One of these pieces had ten or more new segments at the anterior end, which was, no doubt, a heteromorphic tail. V, (one piece) with 17 or more posterior segments and several new anterior segments.

Febr. 6. I, (all dead). II, (2 pieces) one had two and a half new posterior segments, the other three. III, (3 pieces) one had four new posterior segments, one seven, and another two. The last piece had a small new anterior end. IV, (3 pieces) one had fifteen new posterior segments, another had fifteen and the other had also fifteen new segments. The last had also a few irregular new anterior segments. V, (one piece) had seven new posterior segments.

Febr. 27. II, (2 pieces) one with four and the other with three new posterior segments. III, (3 pieces) one with five, one with four and one with three new posterior segments. One of these pieces had three new anterior segments. IV, (3 pieces) one with 20, one with 18, one with 19 (or 20) new posterior segments. One piece had a new anterior end. V, (one piece) with nine new posterior segments.

Apr. 3. II, (dead). III, (3 pieces) one with six, one with four, and one with three new anterior segments. One of these pieces had three new anterior segments. IV, (2 pieces) one with twenty-three, one with eighteen new posterior segments. V, (no record, probably all dead).

May 21. III, (dead). IV, (one piece) with fifteen new posterior segments.

Summary. These results covering seven and a half months show certain points of general interest. First the length of time that some of the small pieces remained alive is remarkable, especially as they were kept in a warm room with a temperature between 60° and 70° F. Despite the length of time that they remained alive none of the most posterior pieces, I, produced any new posterior segments. In the pieces, II, anterior to these only three or four new segments were produced after five months. At this time three pieces of the third lot, III, had made only three, four, and five new posterior segments, but in this same lot at an earlier date another piece had made twelve and another eight posterior segments. In the more anterior lot, IV, the greatest number of new segments were made. Eighteen, twenty and more new segments were produced. In fact one piece of only eleven old segments made twenty-two new ones after three months. The most anterior lot, V, made almost as many segments as the last, the difference being probably accidental, or else due to the fewer pieces remaining alive.

None of these pieces produced as many segments as lay behind the posterior end of the piece in the original worms; although had they lived longer some of them might have accomplished this feat. Whether any of the pieces would ever have produced more than this can not be stated, although to judge from other results this does not seem probable.

Several of these pieces produced new material at the anterior end, but in most cases the new part was too short to determine whether a new head or a new tail was produced. In several cases, however, the new part appeared to be a new heteromorphic tail. It would be a point of much interest to determine whether the number of new segments in these heteromorphic tails is connected with the level from which the new part arises; whether, for instance, from a more posterior level a shorter heteromorphic tail is produced than from a more anterior level[1]. If this should be found to be the case, it would show that the same structural (i. e. internal) factor that determines the length of the new posterior othomorphic tail also determines, ceteris paribus, the length of the new anterior heteromorphic tail. In looking over the few scattered notes that I have bearing on this subject, it seems not improbable that such a relation really exists.

These results agree with my earlier ones in showing that a piece near the posterior end often fails to make good the missing posterior segments. In order to determine if this is due to the size of the

---

[1] The pieces having the same number of old segments.

piece, or to some structural peculiarity of this region I cut off longer pieces of the posterior end and removed from them the last ten segments. In the first experiment twenty-five pieces containing 30 to 40 posterior segments were cut off from as many worms, and then from each piece about ten posterior segments were removed. After a month a small new part was present in fifteen pieces but none could be seen in the other two. After nearly two months more, one piece had 7 new segments, one 3 (or 4), and another 2 (or 3). The remaining two had barely any new material at the end. After two months more four pieces had 8, 5, 4, and 2 new segments respectively. After this some of the pieces died, but no new segments were produced by the others.

Almost identical results were obtained when worms were cut in two in the middle and about ten segments cut off from their posterior ends. These pieces also produced, in most cases, only a few posterior segments.

If these results are compared with those in which the posterior cut-surface is at a more anterior level, it will be found that, in the latter case, regeneration is quicker and a larger number of new segments are produced. It is evident, therefore, that some structural peculiarity is present in the posterior ends that limits the regeneration from this region. Since, however, pieces containing more than 10—15 segments produce more readily, and more frequently, the missing posterior ten segments we must also conclude that the smallness of the posterior pieces is also a factor limiting the power of posterior regeneration. The histological character of the most posterior region is somewhat different from the middle region of the worm, and there is present a relatively large number of chlorogogue cells.

Regeneration of Anterior Ends from three Different Levels.

During four winters the following experiment has been carried out. In set *A* the worms were cut as near as possible to the fifteenth segment; in set *B* just behind the girdle (about the 25th segment); in set *C*, through the middle of the worm. The posterior pieces were kept in order to find out what sort of structure would develop at the anterior end. When cut at the 15th segment, the gizzard is left exposed in most cases at the anterior end; when cut at the girdle or in the middle, the stomach-intestine is exposed. In those cases in which a head (composed of 5, or fewer segments) developed at the anterior end I wished to see what part of the digestive tract was produced in the new head, and how the connection with the old part was made. Some of the worms were kept for a long time to see if new reproductive organs would develop, since these were entirely removed with the old anterior piece.

In all 335 worms were operated upon. The year and the number of worms is as follows:

	A	*B*	*C*
'97	25	15	25
'98	30	30	30
'99	30	50	50
'01	25	0	25
	110	95	130

The best series is that of '98 which I select as typical.

The worms were cut in two on Oct. 10. On Jan. 15 there were 27 worms alive in *A*. They had, or most of them had, short knob-like outgrowths (not so long as in *C*). In *B*, 26 worms were alive. About half of them had a new outgrowth. In *C* there were 30 worms of which about half had each a new tail-like outgrowth having from a few to 20 new segments.

On Feb. 26 there were alive in *A*, 20 worms of which a distinct new head of 2 or 3 segments was present in 6, in 10 the new part was probably a head; and in 4 no regeneration had taken place. In *B* there were 36 pieces[1]) of which 14 were doubtful (probably more were heads than tails), 1 had a distinct new head, 5 had a new heteromorphic tail, and 12 showed no regeneration. In *C* there were 31 pieces of which 14 had new heteromorphic tails (from 5 to 25 new segments), 2 were doubtful, and 15 had not regenerated.

On April 1 there were 13 worms alive in *A*, of which 3 had a new head, 4 were doubtful, 1 had possibly a new tail, and 5 had not regenerated at all. In *B* there were 28 worms of which 10 had new tails, 8 were so abnormal that the nature of the new structure was doubtful, and 10 had not regenerated at all. In *C* there were 22 pieces, of which 8 had new heteromorphic tails, 1 was doubtful, and 13 had not regenerated.

On Apr. 21 only 2 worms were alive in *A* (one with a new head of two segments); in *B*, 11 were alive of which 3 had new hetero-

---

[1]) Some of the worms must have broken in two. The discrepency was not noted at the time and the pieces not examined to see if this was the case.

2. The position of the nervous system in the three oblique cuts is indicated in Fig. 36, 37, 38. In Fig. 36 the cut end of the nervous system is in the middle of the posterior oblique surface; in Fig. 37 it lies at the outer, pointed, ventral end; in Fig. 38 at the inner ventral end of the cut-surface.

In the first case, Fig. 36, the new part (head or tail, more often the latter in these experiments) grew out at right angles to the cut-surface with the old nerve-cord as the center of the new part. In the second case, Fig. 37, the new part extends backwards from the outer end standing also more or less vertically at right angles to the cut-surface. The new part does not arise from the middle of the cut-surface, as in the last case, but the new tail arises nearer the outer end, with the end of the old nervous system as the point of departure. The upper parts of the more proximal segments complete themselves and take no direct part in the formation of the new tail. In the third case, Fig. 38, the conditions are peculiar and often an abnormal development takes place. It can be seen that the new tail is formed at the region in which the old nervous system ends, that it stands at right angles to the cut-surface, and that it does not occupy the entire cut-surface. The connection between the old segments of the outer part of the oblique surface and the new segments is generally very irregular and incomplete. A common form of abnormality which occurs quite often in the new tail is shown in Figs. 26, 27, 28, 29, 31. It is clear that the result is due to the lack of development of the dorsal surface of the new part, so that the anal opening instead of being carried out to the end remains near the upper part of the cut-surface Figs. 26, 28, 31. A more extreme case is shown in Fig. 29. Here the ventral wall alone has developed, so that the new part has the form of a concave plate. The digestive tract protrudes in folds from the upper surface of the plate. The plate contains the nervous system along the mid-ventral line, the nephridia at the sides and a terminal growing zone, where new segments are added. The plate is metamerically segmented. There can be no doubt that these kinds of abnormalities are due to the lack of development of the dorsal wall of the new part. Regeneration has begun at the ventral surface where the cut-end of the nerve-cord is present. A growing zone has been formed at the end of the new part. New segments, or the ventral part of segments, are added as in ordinary regeneration. In consequence of the lack of development of the dorsal side the new part may be thrown into a spiral like that of Fig. 27.

At present I can not state with certainty to what this lack of development of the dorsal side is due. It may be due to failure of the new and the old parts to form a morphological union; or it may be due to the way in which the digestive tract forms an opening to the exterior, or to the degeneration of the overhanging old dorsal wall, while the ventral wall is regenerating. The abnormality is most often found — it is at best of rare occurence in the extreme form shown in these figures — in worms that have been cut off as in Fig. 38. It also occurs when the cut is made laterally as in Fig. 36, but as the oblique cut may also extend forward on the ventral surface such instances may be comparable to the last. In a few cases I have obtained similar results, when a piece was cut out of the mid-ventral wall at the posterior end of a piece.

When the oblique cut like that of Fig. 38 is near the anterior end a new head arises somewhat on the ventral side as shown in Fig. 32. The failure of the new and the old segments to properly unite is obvious in this figure.

A curious result was obtained in one case in which a worm had been cut in two at about the middle by an oblique cut with the anterior end of the cut on the dorsal side. The cut-surface was like that of Fig. 38, but the regeneration of which I am about to speak took place from the anterior end of the posterior half of the worm. As shown in Figs. 39, 40, two small knobs appear near the end (on the ventral side) of the oblique cut. They lie to the right and left of the middle line. When cut into sections it was found that a prolongation of the blind anterior end of the digestive tract extends to each of these projections, but not into them. The projections were solid, being filled with material that in continuous with the two layers of muscles of the body-wall. Ectoderm covered the surface. The old nervous system ends abruptly three or four somites behind the level of these projections. The anterior end of the cord plunges here into the body wall, and its fibres can be traced forward for a short distance between the two muscle-layers. There can be no doubt that the two projections of the digestive tract are connected with the formation of the two knobs, and it is probable that they represent an abortive attempt to form new heads or more probably heteromorphic tails. This result is especially interesting in connection with the results described in the first part of this paper, where on several occasions it was noted that a small projection was present at the anterior end of the worm, even when the nervous system did not extend into this region, Figs. 3, 6, 11. It was also recorded that these projections were found in connection with the anterior end of digestive tract. In the light of these results, I think it is probable that the presence of the anterior end of the digestive tract near a regenerated part of the body-wall acts as a stimulus causing an outgrowth in that region, but in the absence of the nervous system the new part fails to become organised to form a head.

Regeneration of Lumbriculus.

There is a peculiar relation connected with the non-sexual propagation of lumbriculus to which, I believe, attention has not been especially called. It had been noticed by earlier observers that the number of anterior segments produced by pieces is limited in much the same way as in the earthworm. In a species that I examined I found that not more than six or seven new anterior segments generally come back. The pieces are therefore limited in their anterior growth. If the pieces were also limited in their posterior growth to the same number of segments that lay behind that piece in the original worm (as seems to be the case in the earthworm), then the new worms (except the one from the anterior piece) would be shorter than the original individual according to the position in the original worm from which the pieces were taken. If this were the case, and if then the new worms (except the anterior one) should again propagate themselves by division the second generation would be correspondingly shortened (except the one from the head end) and if we imagine the process to continue most of the worms would ultimately be reduced to a very few segments, theoretically to a single one. Since we do not find this to be the case in nature we can only suppose that the posterior end of lumbriculus has an unlimited growth; at least this is the case if the worms propagate for any length of time by means of the non-sexual-process. It is notorious that the sexual forms of this worm have been rarely found, so that it is not improbable that non-sexual propagation does go on for a long time. The result of such a process would be that almost the entire race would become a regeneration product. The few worms that have come from the more anterior regions of the body in which the sexual organs are believed to develop, would be few in comparison to the others, and in the course of time they might be entirely eliminated by accidents. From this discussion we can see that two possibilities exist in relation to the formation of sexual forms. First, the majority of worms that have arisen from regenerated regions of some of the original worms might be incapable of producing sexual organs. Since they produce anteriorly only the head segments. Only the few worms that contained the genital segments of the original worm would be capable of producing the sexual cells. Second, all the new worms might produce sexual organs in the new segments in which case the sexual cells would arise from somatic cells that have themselves been regenerated. If sexual cells arose in this way and embryos were produced then the chain of non-sexual forms would be broken. If only some of the worms propagated sexually both methods of reproduction might go on at the same time. Until more facts are known in respect to the sexual reproduction of lumbriculus, we can not decide which of these possibilities is realized.

These ideas were suggested by some experiments on lumbriculus which I made during the winter of '99–00, but the results are too fragmentary to give in extenso. I found that short anterior pieces of from 10–15 segments usually die, as BONNET and others have also observed. Anterior pieces under 12 segments rarely produced new posterior ends, and even pieces that had begun to regenerate often died. On the other hand short pieces of only 4 or 5 segments from the middle of the body produced new segments at both ends. Pieces from the posterior region often died, and although I did not attempt to determine the exact limits of regenerative power in this region, yet it appeared that many segments must be present in order that regeneration may take place, but possibly this may depend on whether the posterior end has recently regenerated.

In several cases the worms were cut into a number of pieces and the pieces kept apart and numbered according to their position in the original worm. After several months the new worms were killed, and the number of old segments and the new ones at the ends were counted. B^1 contained the old head, B^2 was the next piece behind this, etc.

	Segments in New Head	Segments in Old Part	Segments in New Tail
B^1	—	17	81
B^2	$6^1/_2$	19	108
B^3	6	30	119
B^4	7	20	94
B^5	7	12	28

	Segments in New Head	Segments in Old Part	Segments in New Tail
D^1	—	dead	—
D^2	6	13	89
D^3	7	16	81
D^4	7	15	96
D^5	7	10	68
D^6	7	18	111
D^7	7	9	60

These tables show in the first place that the number of new segments produced at the posterior end bears no relation to the region from which the regeneration takes place, but rather to the number of old segments in the piece. In as much as I failed to record the number of segments in the posterior pieces that were rejected it can not be stated whether the highest number of segments produced by the pieces — especially by the posterior pieces — is greater than the number that lay originally behind that region; but judging from other cases in which the total number of segments were recorded it appears that in none of these cases had as yet the full compliment of posterior segments been produced.

The number of anterior segments produced by these pieces was, in all but two cases, seven, and the number produced bore no relation to the region from which the piece had come. In another experiment this point was tested for quite short pieces. The results are as follows.

No. of Old Segments	No. of New Anterior Segments	No. of Old Segments	No. of New Anterior Segments
8	7	12	6
2	6	6	6 (or 7?)
6	7	19	7
12 +	6 +	$4^1/_2$	5 + (?)

These figures show that the number of anterior segments is not determined by the size of the old piece.

In other cases I removed one to six segments from the anterior end of several worms. In these cases the number cut off generally comes back. This result agrees with that on the earthworm. In a few other cases, the head end of a worm was split in two lengthwise and one half removed. In those in which the anterior half that remained did not slough off, it regenerated laterally.

Summary.

1) When a piece of the mid-ventral wall of the earthworm is cut out, as shown in Fig. 33, (a few of the anterior segments having been previously removed), the lateral edges of the region come together and unite. The anterior end of the ventral nerve-cord is removed with the piece of the wall. At the anterior end of the worm all the longitudinal organs are present except the nerve-cord. Owing to the absence of the latter a new head does not regenerate at the anterior end. At the level at which the old nerve-cord ends a new head grows out in the mid-ventral line, as shown in Figs. 2, 10, 11. The new head is composed of a few segments. It may or may not contain a digestive tract. Whether it has a digestive tract, or not depends most probably upon whether the old digestive tract has been injured during the operation of removing the piece of the mid-ventral wall. The new head contains in all cases a ventral cord which lies inside the body wall, and arises from the anterior end of the old nerve-cord, Fig. 25. At the distal end the new nerve-cord in the new head forms connectives and a brain if a digestive tract is present, but if none has been formed the ventral cord terminates without forming a separate brain and connectives, although an enlargement at the end may represent these structures. The body wall is made up of the characteristic layers, Fig. 24; although the setae are fewer than normal. Septa are absent but irregular strands may represent them. Nephridia seem also to be absent.

2) If after removing, as before, a few anterior segments a cut is made to one side of the mid-ventral line (as shown by the heavy line in Fig. 34) and then a piece is removed (beginning at the posterior end of the cut) from the mid-ventral line, Fig. 34, two anterior ends of the nerve-cord will be left, one at the anterior cut-surface, and the other at the posterior end of the region from which the piece of the ventral wall was removed. Under these circumstances two heads may develop (one or the other may fail at times to do so); one of which will be a large head at the anterior end and the other a small head from the mid-ventral line near the ending of the main ventral cord. The latter may or may not contain a digestive tract (connecting with the main one) and resembles the head in the last experiment which arises under similar conditions.

3) The two preceding experiments show that in order to form a new head it is necessary that a cut-end of the nerve-cord is pre-

sent, and that there is no necessity for the presence of a digestive tract. This result gives us a first step in the analysis of the internal factors necessary for the formation of a new head in the earthworm.

4) The two preceding experiments indicate with some probability that bifid tails, which are sometimes found in annelids, Fig. 30, arise from injuries involving the old ventral cord.

5) If pieces of 10 to 15 segments each are cut from the posterior end beginning at the distal end it will be found that the second piece (10 segments from the posterior end) often fails to produce new segments at its posterior end, and at most produces only a few new ones. Passing forward the tendency to produce new posterior ends is more marked towards the middle of the worm. None of the pieces produce more segments than lay behind the region of the original worm from which the piece came.

6) Many of these pieces produce heteromorphic tails at the anterior end. It is probable that the number of segments in these heteromorphic tails is regulated by the region from which the tail arises. The nearer the region from which the heteromorphic tail develops is to the posterior end, the fewer the number of heteromorphic segments, and, vice versa, the further from the posterior end the greater the number of segments.

7) Longer pieces (than those used in 5) near the posterior end produce more often the missing segments, although regeneration is conspicuously less marked near the posterior end than at anterior levels.

8) When the anterior 15 segments are removed (the worm being cut through at the level of the gizzard) a new head is sometimes produced, but it is generally composed of a few segments, which are often abnormal. When the worm is cut in two at the level of the girdle a new head is still less often regenerated although abnormal heads may sometimes appear. When the worm is cut in two in the middle there develops on the anterior end of the posterior half a heteromorphic tail.

9) In the first two of the preceding (8) three categories, it was found in most cases examined which had produced new heads that the new stomodoeal invagination did not join the old digestive tract, but ended posteriorly blindly. A brain and connectives were present.

10) When worms are cut in two by oblique lateral cuts (Fig. 36) the new part arises in the middle of the oblique surface (where the nerve-cord ends). When cut off obliquely with the anterior end of

the oblique plane on the dorsal side (Fig. 37) the new part arises near the ventral end of the oblique surface (where the nerve-cord ends). When the oblique cut is made with the anterior end on the ventral surface (Fig. 38) the new part arises near the ventral surface (again where the nerve-cord ends). These results show that the nerve-cord is the center around which the new part is formed, and the obliquity of the cut plays a subordinate role in the result.

11) In the last case just cited, it is not infrequent to find that only the ventral and lateral body walls are regenerated, Figs. 26, 29, 31. The anus lies, therefore, on the dorsal and anterior end of the new part. In consequence of the absence of the dorsal surface of the new part, abnormalities like those shown in Figs. 26, 27, 28, 29, 31, result.

Zusammenfassung.

1) Wird ein Stück aus der Mitte der ventralen Körperwand des Regenwurms herausgeschnitten, wie in Fig. 33 (einige vordere Segmente wurden vorher entfernt), so schließen sich die seitlichen Ränder der Gegend zusammen und verschmelzen. Das vordere Ende des ventralen Nervenstrangs wurde mit dem Wandstück entfernt. Am Vorderende des Wurms sind alle Longitudinalorgane erhalten mit Ausnahme des Nervenstrangs. Wegen der Abwesenheit des letzteren entwickelt sich kein neuer Kopf am Vorderende. In derselben Höhe, in der der alte Nervenstrang endet, keimt ein neuer Kopf in der ventralen Mittellinie, wie die Fig. 2, 10, 11 zeigen. Das neue Haupt ist aus einigen Segmenten zusammengesetzt. Es kann einen Verdauungskanal enthalten, oder auch nicht. Beide Möglichkeiten hängen höchst wahrscheinlich davon ab, ob der alte Verdauungskanal während der Operation zur Entfernung des ventralen Wandungsmittelstückes verletzt wurde. Der neue Kopf enthält allfällig einen nervösen Bauchstrang, der nach innen von der Körperwand liegt und vom vorderen Ende des alten Nervenstranges entspringt (Fig. 25). Am distalen Ende bildet der Nervenstrang in dem neuen Kopfe im Falle des Vorhandenseins eines Verdauungskanals Konnektivzweige und ein Gehirn, andernfalls endigt der Bauchstrang ohne die Bildung eines besonderen Gehirns und solcher Konnektivzweige, obgleich eine Endanschwellung diese Bildungen vertreten kann. Die Körperwand besteht aus den typischen Schichten (Fig. 24); allerdings sind die Borsten seltener als normal. Septa fehlen, können aber durch unregelmäßige Zellstränge vertreten werden. Nephridien scheinen gleichfalls zu fehlen.

2) Wenn nach der wie oben erfolgten Entfernung einiger vorderer Segmente ein Schnitt seitwärts gegen die ventrale Mittellinie geführt wird (entsprechend der dick gezeichneten Linie in Fig. 34) und dann ein Stück (angefangen vom hinteren Ende des Schnitts) von der ventralen Mittellinie (Fig. 34) entfernt wird, so bleiben zwei vordere Enden des Nervenstrangs übrig, eins an der vorderen Schnittfläche und das andere hinten an der Region, die dem entfernten Bauchwandstück entspricht. Unter diesen Umständen können sich zwei Köpfe entwickeln (der eine oder der andere kann dabei aber auch versagen),

von denen der eine, und zwar der am Vorderende, ein großer Kopf wird, der andere, nämlich der an der Mittellinie nahe dem Ende des Haupt-Bauchnervenstranges, ein kleiner. Der letztere kann entweder einen Verdauungskanal (der sich dann mit dem Hauptermährungskanal verbindet) enthalten, oder auch nicht, und ähnelt dem beim vorigen Versuch unter ähnlichen Verhältnissen entstandenen Kopfe.

3) Die beiden vorerwähnten Versuche zeigen, dass zur Entstehung eines neuen Kopfes das Vorhandensein eines Schnittendes des Nervenstranges nothwendig ist und dass dafür das Vorhandensein eines Ernährungskanals nicht nöthig ist. Dieses Resultat bedeutet den ersten Schritt in der Analyse der inneren Bedingungen, welche zur Neubildung eines Kopfes beim Regenwurm nothwendig sind.

4) Die beiden vorigen Versuche machen es bis zu einem gewissen Grade wahrscheinlich, dass zwei gespaltene Schwänze, welche bisweilen an Anneliden (Fig. 30) entstehen, sich von Verletzungen herleiten, welche den ursprünglichen Bauchstrang betrafen.

5) Werden Stücke von je 10—15 Segmenten vom Schwanz abgeschnitten indem vom Hinterende angefangen wird, so findet man öfter Unfähigkeit des zweiten Stückes (10 Segmente vom Hinterende) nach hinten zu neue Segmente hervorzubringen und es kommt meist nur zur Bildung einiger weniger neuer. Kommt man weiter vorwärts, so wird die Tendenz zur Bildung neuer Hinterenden deutlicher nach der Mitte des Wurms zu. Kein Stück bringt eine größere Anzahl Segmente hervor, als im ursprünglichen Wurm hinter der Stelle lagen, von der das Stück kam.

6) Manche von diesen Stücken bringen am Vorderende heteromorphische Schwänze hervor. Möglicher Weise übt die Ursprungsgegend des Schwanzes einen regulirenden Einfluss auf die Anzahl der zu ihm werdenden Glieder aus. Je näher die Ursprungsstelle des heteromorphischen Schwanzes dem Hinterende liegt, desto geringer ist die Anzahl heteromorphischer Segmente, und, umgekehrt, je weiter nach vorn vom Hinterende, desto größer ist seine Gliederzahl.

7) Längere Stücke (als die unter 5) verwendeten) in der Nähe des Hinterendes ergänzen öfter die fehlenden Segmente, obwohl Regeneration ganz deutlich in der Nähe des Hinterendes weniger ausgesprochen erscheint, als in weiter vorn liegenden Querschnittsgebieten.

8) Werden die vorderen 15 Segmente entfernt (wobei der Wurm in der Höhe des Magens durchschnitten wird), so entsteht bisweilen ein neuer Kopf, der aber gewöhnlich nur aus ein paar Segmenten besteht, die oft abnormal sind. Selbst wenn der Wurm in der Höhe seines Gürtels durchschnitten wird, ist die Regeneration eines normalen Hauptes noch weniger häufig, obwohl abnormale Kopfbildung bisweilen auftreten kann. Wird der Wurm in der Mitte entzwei geschnitten, so entwickelt sich am Vorderende der Hinterhälfte ein heteromorphischer Schwanz.

9) Bei den ersten beiden, der unter 8) erwähnten drei Kategorien ergiebt die Prüfung derjenigen Fälle, in denen neue Köpfe gebildet wurden, dass die neue Munddarmeinstülpung nicht immer mit dem ursprünglich vorhandenen Verdauungstrakt in Verbindung tritt, sondern nach hinten zu blind endigt. Gehirn und Konnektivnerven waren vorhanden.

10) Werden Würmer durch schräge Seitenschnitte zertheilt (Fig. 36), so erhebt sich der neu gebildete Theil in der Mitte der schrägen Schnittfläche (wo der Nervenstrang endet). Wird der Schnitt so geführt, dass der Vorderrand

der Schnittfläche dorsal liegt (Fig. 37), so erhebt sich die Neubildung nahe dem ventralen Ende der schrägen Schnittfläche (wo der Nervenstrang endet). Wird endlich der Schnitt so geführt, dass der Vorderrand der Schnittfläche ventral liegt (Fig. 38), so entsteht die Neubildung dicht am Rande der Bauchfläche (wieder, wo der Nervenstrang endet). Daraus scheint hervorzugehen, dass der Nervenstrang ein Centrum darstellt, um welches sich die Neubildungen gruppiren, und dass die Schräge des Schnittes dabei eine nur untergeordnete Rolle spielt.

11) Im letztcitirten Falle findet man nicht gerade selten, dass nur die ventrale und laterale Körperwand sich regenerirt (Fig. 26, 29, 31). Der Anus liegt daher auf dem Rücken nach vorn zu in dem neugebildeten Theil. Als Folge der Abwesenheit der eigentlichen Rückenwand der Neubildung ergeben sich abnorme Bildungen, wie die in Fig. 26—29, 31 gezeichneten.

Fig. 1.

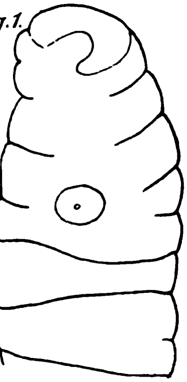


Fig. 2.

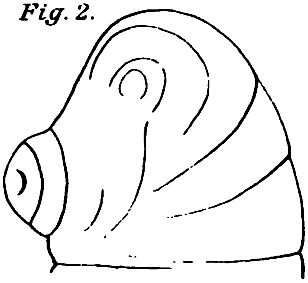


Fig. 3.

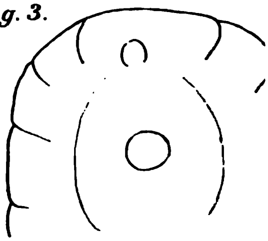


Fig. 4.

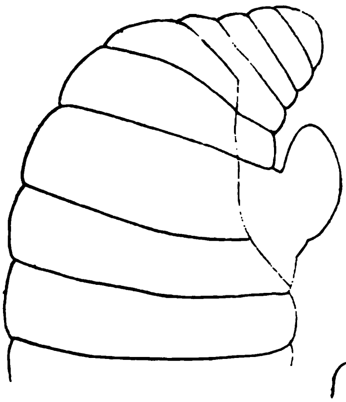


Fig. 5.

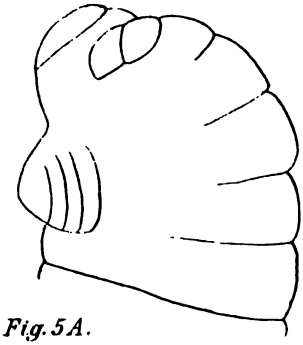


Fig. 6.

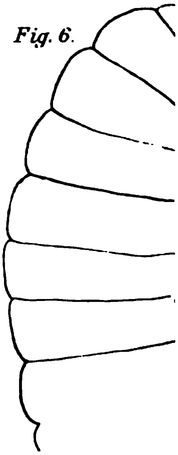


Fig. 5 A.

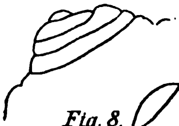


Fig. 8.

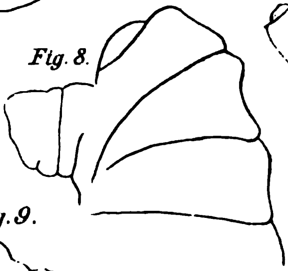


Fig. 10.

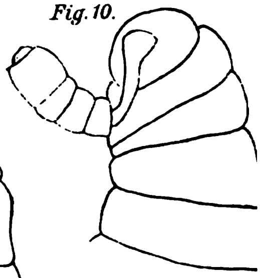


Fig. 7.

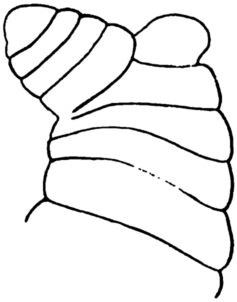


Fig. 9.

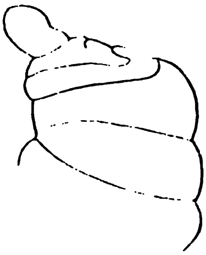
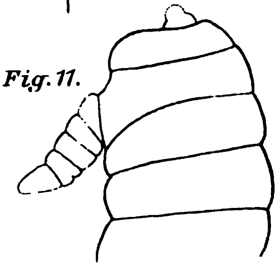


Fig. 11.



T. H. M. del.

12.

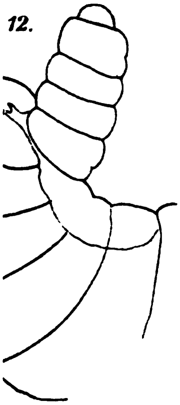


Fig. 13.

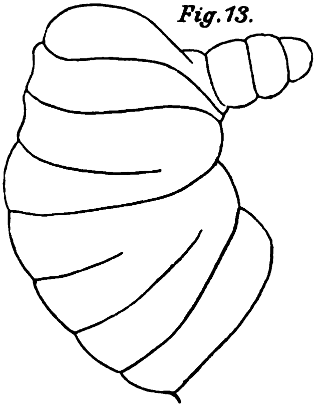
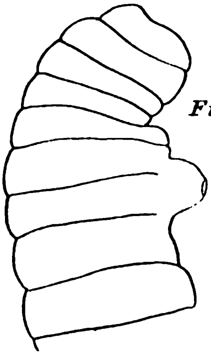


Fig. 14.



15.

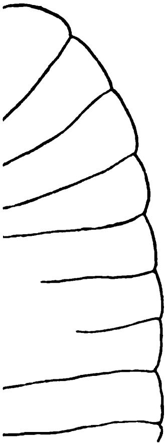


Fig. 16.

Fig. 16 A.

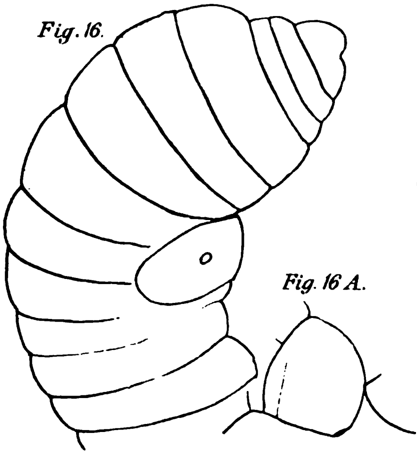
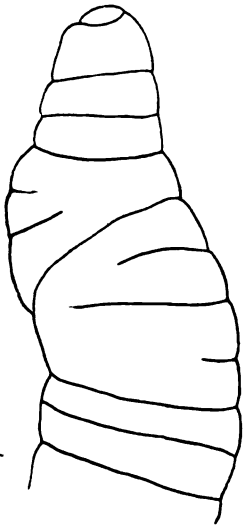


Fig. 17.



'ig. 18.

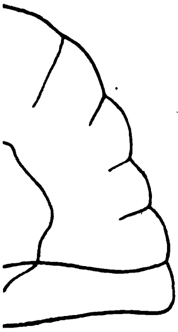


Fig. 19.

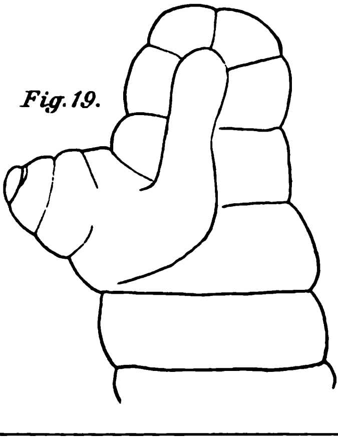
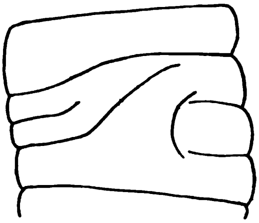


Fig. 20.



Lith. Anst. Julius Klinkhardt, Leipzig.

.eipzig.

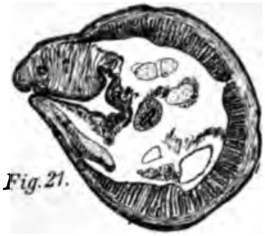


Fig. 21.

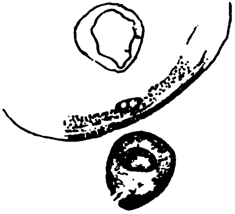


Fig. 22.

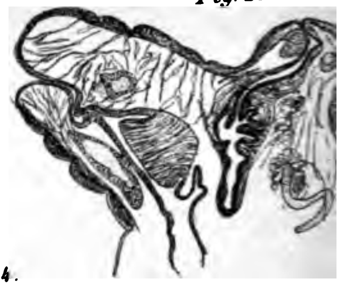


Fig. 23.

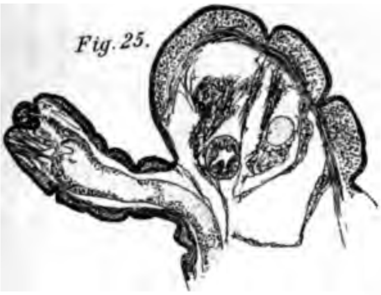


Fig. 25.

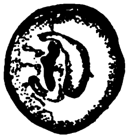
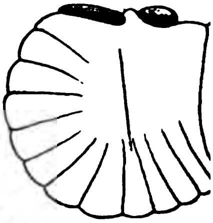


Fig. 24.



Fig

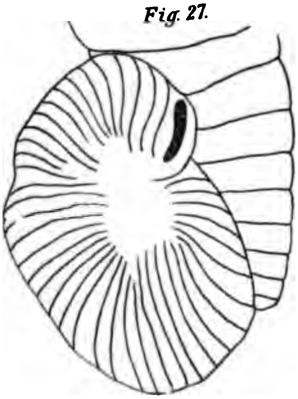


Fig. 27.

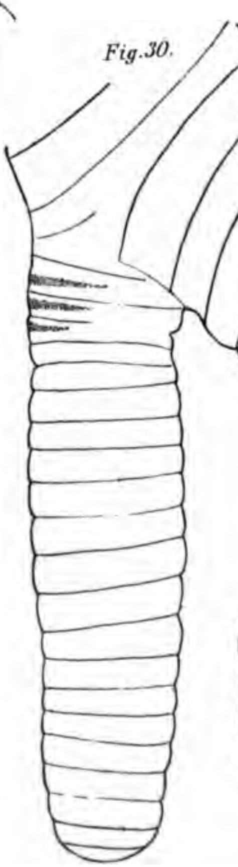


Fig. 30.

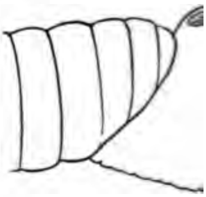


Fig. 28.

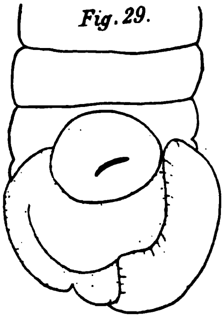


Fig. 29.

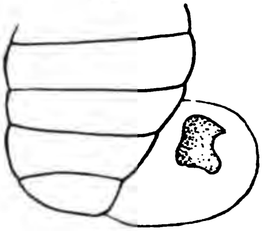


Fig. 31.

T.H.M. del.

Fig. 33.

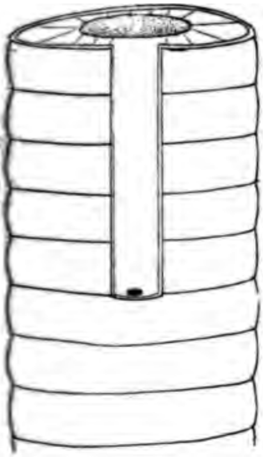


Fig. 34.

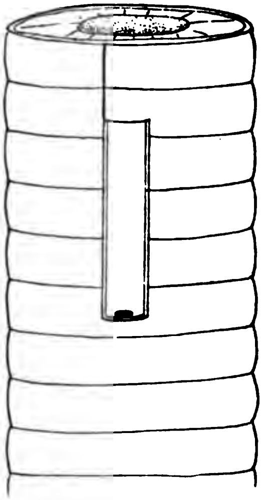


Fig. 35.

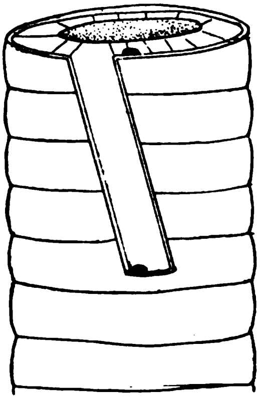


Fig 36.

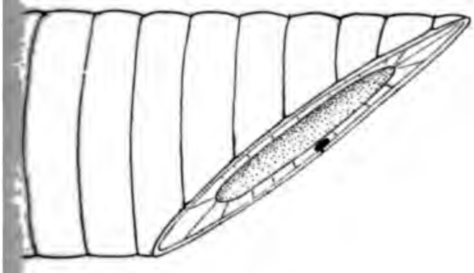


Fig. 37.

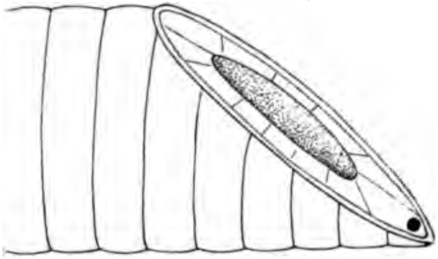


Fig. 38.

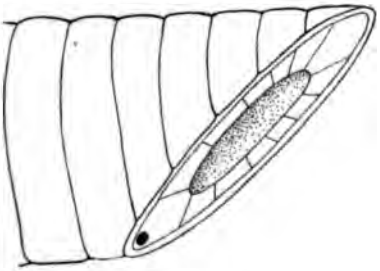


Fig. 39.

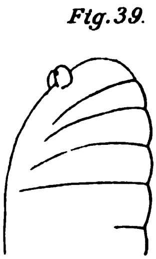
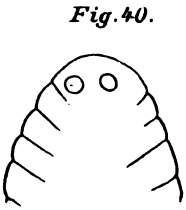


Fig. 40.



U of M

Lith. Anst. Julius Klinkhardt, Leipzig

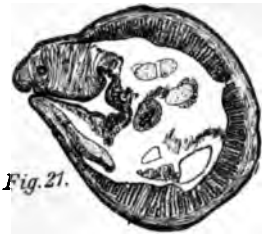


Fig. 21.

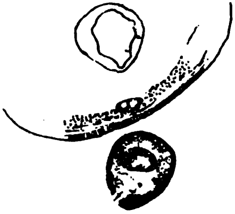
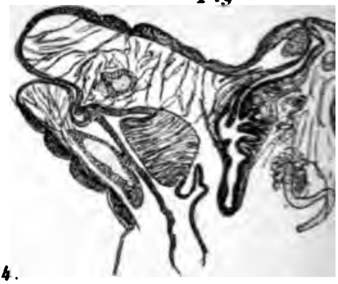
Fig. 22.


Fig. 23.


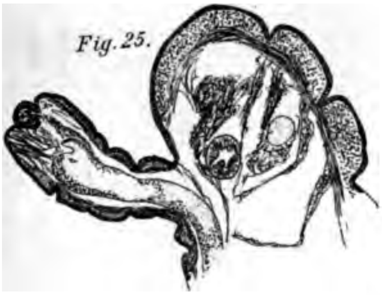
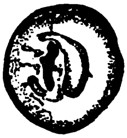


Fig. 25.

Fig. 24.


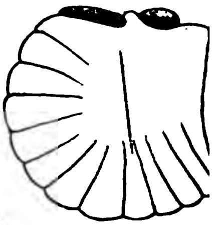
Fi


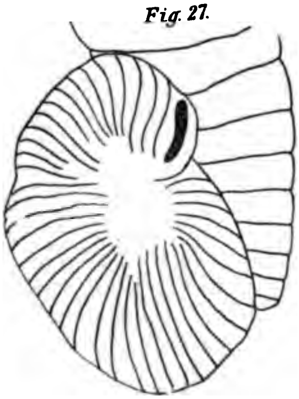
Fig. 27.


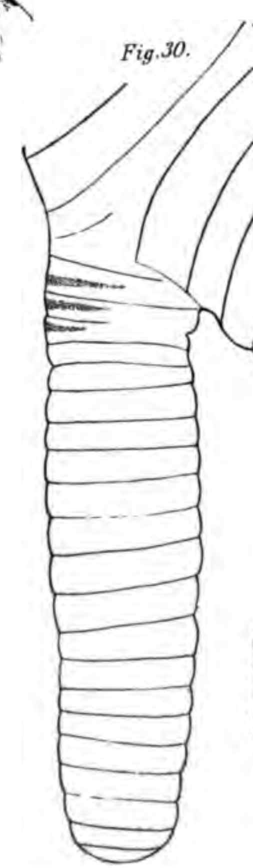
Fig. 30.


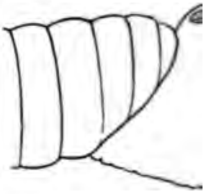
Fig. 28


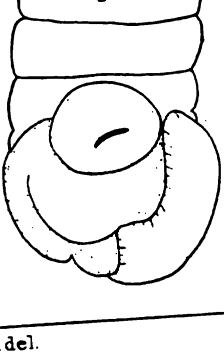
Fig. 29.


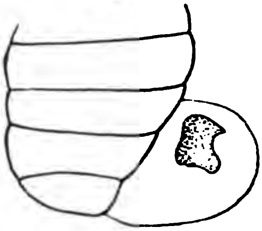
Fig. 31.


Fig. 33.

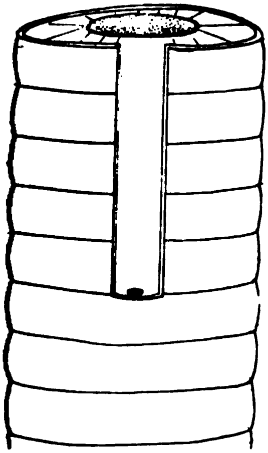


Fig. 34.

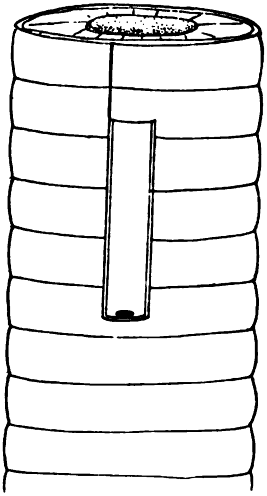


Fig. 35.

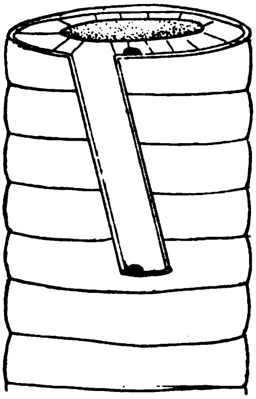


Fig 36.

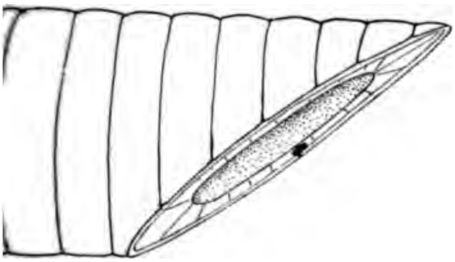


Fig. 37.

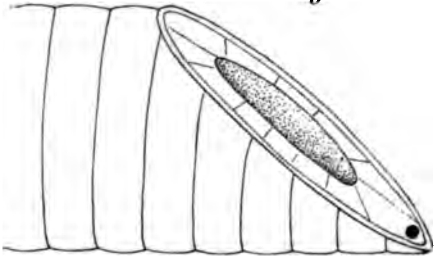


Fig. 38.

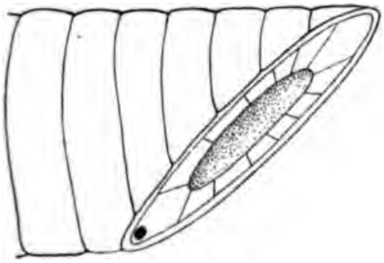


Fig. 39.

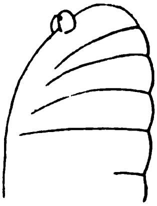
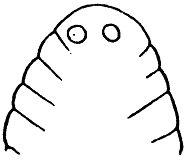


Fig. 40.



UofM

Lith. Anst. Julius Klinkhardt, Leipzig

nn in Leipzig.

Regeneration in Tubularia mesembryanthemum.

By

N. M. Stevens.

II.

With 13 figures in text.

Eingegangen am 13. Juni 1902.

In October 1901, when my paper on Tubularia went to press, these hydroids were very small and pale at Naples. In December and January the material was in normal condition, and I was able to make some further observations on the behavior of the red pigment granules in regenerating pieces.

In the larger, brighter colored specimens, the ejection of a large pigment ball by the new hydranth is, in very many cases, as easily observed as in the Woods Hole species; moreover, the same phenomenon occurs in the white variety, the pigment mass being a pale yellow and easily identified with the pigment granules in the endoderm of the hydranth.

In several specimens, the mouth opening was formed and the pigment thrown out while the hydroids were being observed with the low power of a compound microscope. The pigment is most often ejected in one mass, soon after the hydranth emerges from the tube, but may be removed in smaller masses at intervals extending over several hours. I have seen specimens try for hours to rid themselves of masses which had adhered to the wall of the pharynx and could not easily be dislodged.

Occasionally, instead of collecting at the distal end of the tube, the pigment may be observed lying on the bottom of the tube scattered along its whole length, or it may collect at the proximal end of the piece. In one piece the pigment mass, when first observed was at a bend in the tube about a centimeter behind the hydranth which was already expanded; a few minutes later it went up into the proboscis, but was not ejected; and two hours later it was seen in the foot, which was forming at the proximal end.

In many pieces the pigment ball was seen forming at the distal end before and during the development of the tentacle anlagen, particles from the circulating fluid being gradually added to the mass (Fig. *A*).

The different shades and colors of Tubularia seem to be due not so much to presence or absence of pigment granules as to difference in shade or color of those granules. The hydranths of the white variety contain a large amount of pigment, but it is of a pale yellow color and therefore inconspicuous. The pigment in growing parts seems to be of a deeper shade as well as more abundant than in older tissue: this may be observed in the foot which is often developed at the proximal end of a long piece, as well as in new hydranths. All the evidence seems to indicate formation of new pigment in new parts and rejection of that which has come from degenerated parts, as waste material.

In discussing the matter, Dr. DRIESCH called my attention to the case which suggested to him the idea that a quantitative relation existed between the number of red granules in the circulation and the amount of red pigment in the endoderm of the new hydranth. A piece which had been cut between the anlagen of the proximal and distal tentacles, showed, after some hours, degeneration of the proximal tentacle anlagen, and a pigment ball at the distal end. Several hours later the pigment ball had disappeared and new tentacle anlagen, both distal and proximal, had appeared, — longitudinal stripes red with pigment. The natural interpretation of the phenomena observed was that the red granules that had vanished from the end of the tube, had gone into the new tentacle anlagen (DRIESCH '99).

As my observations on the normal regeneration of a hydranth made the correctness of this interpretation seem improbable, I repeated DRIESCH's experiment. In four lots of Tubularia, from which the distal tentacle anlagen were cut, Jan. 17th, 18th and 19th, all the classes of regeneration described by DRIESCH ('99) for such cases, except the one bearing on the point in question, were found.

Jan. 27th, a large number of specimens were cut, and out of one lot of twelve there were five that showed degeneration of proximal tentacle anlagen and formation of new proximal and distal tentacles.

In all of these cases the anlagen were only just visible when the distal end was cut off; when they are well advanced, they seem always to go on developing instead of degenerating. All of these specimens showed a large pigment ball at the anterior end after the degeneration of the tentacle anlagen: in one case the ball broke up and went into the circulation before the appearance of the new tentacle anlagen, but reappeared later and was ejected by the hydranth; in the other four cases it remained at the distal end during the formation of the anlagen, and the whole development of the hydranth and was ejected as before described (STEVENS '01, Fig. *A*). Moreover, in one long piece, a new hydranth had developed, meanwhile, at the proximal end and pigment was also ejected there.

Fig. *A*.

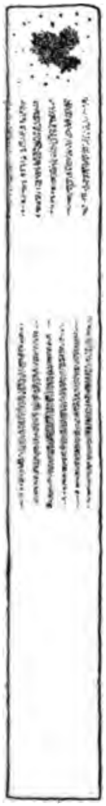


Fig. *B*.

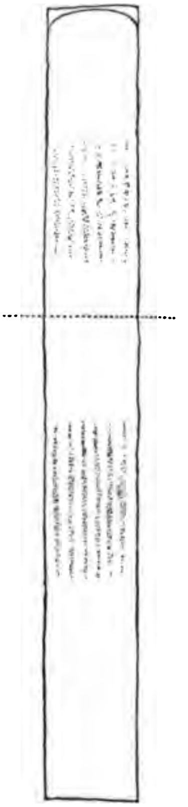


Fig. *C*.

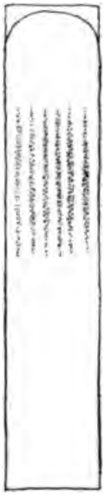


Fig. *D*.

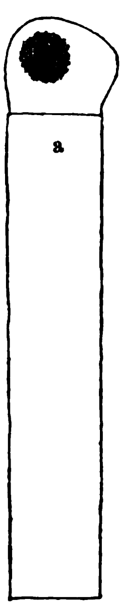


Fig. *E*.

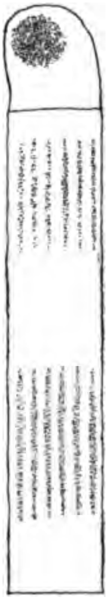


Fig. *A*. Specimen in which the pigment was seen collecting at the distal end while the tentacle anlagen were forming.

Fig. *B*. Specimen cut between the distal and proximal tentacle anlagen, at an early stage of their development, 9 30 A. M., Jan. 27th.

Fig. *C*. Appearance of the same at 4 00 P. M.

Fig. *D*. - - - - - 8 30 A. M. Jan. 28.

Fig. *E*. - - - - - 4 00 P. M. - -

Fig. *B* shows a sketch of one of these specimens cut as indicated at 8 30 A. M., Jan. 27th. At 4 00 P. M. the anlagen appeared unchanged (Fig. *C*). At 8 30 the next morning, the anlagen had disappeared and a ball of pigment was observed at the distal end (Fig. *D*).

The specimens were kept under observation during the day, and at 4 00 P. M. new anlagen had appeared in all five, the pigment mass remaining at the distal end during this whole period of observation in every case but one, as noted above (Fig. *E*). The next morning the hydranths all came out and the pigment was ejected as usual. These results would seem to show conclusively that the red pigment granules and masses seen in the circulating fluid of regenerating pieces of Tubularia are waste material.

Closing in of the cut end. — This phenomenon has been described by MORGAN ('01) as a drawing in of the coenosarc from all sides, constantly narrowing a circular opening until the center is reached and the opening closed. In a recent paper ('02), he says that the opening is not closed by a bending over of the cut edge of the coenosarc toward the center, but that the closing membrane is largely a new formation, which begins a short distance back from the cut end. He attributes the method of closing to a new kind of contractile force, exerted by the tissue as a whole rather than by individual cells.

My observations both at Woods Hole on Tubularia crocea, and at Naples on Tubularia mesembryanthemum led me to somewhat different conclusions.

The cut edge seems to bend over slightly toward the center, the cells meanwhile coming into line and forming a smooth edge, which moves straight toward the center parallel with the plane of the cut. One would hardly expect to find necrotic tissue in a cut surface of Tubularia, but to find the cut cells rounding up as they do in pieces of Protozoa. Sections show that the ectoderm and endoderm take part equally in the closing-in process, both extend to the circular edge in all stages; but the ectoderm clings to the perisarc and loosens its hold less rapidly than the edge moves toward the center, giving a slightly concave appearance to an optical section of a closing or closed end (Figs. *F*, *G* and *M*). As one watches the closing, these peripheral ectoderm cells are seen, hanging back, as it were, but not left behind, for they keep their place, relative to the other cells of the coenosarc, and are gradually pulled away from the perisarc by the force which is carrying the edge toward the center. If, as

Morgan says ('02), the closing membrane were a new formation, beginning somewhat back from the cut edge, without any bending over of that edge, it would necessarily be formed from endoderm alone; both observation of the closing end and examination of sections show that this is not the case, but that the membrane consists of a layer of endoderm and a layer of ectoderm, both extending to the edge in all stages (Fig. *M*).

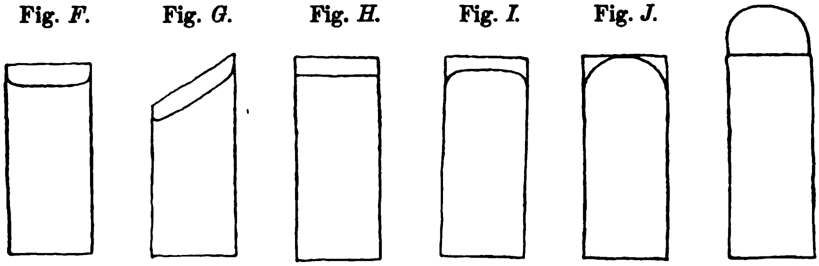


Fig. *F*. Fig. *G*. Fig. *H*. Fig. *I*. Fig. *J*. Fig. *K*.

Figs. *F* to *K*. Closing and later behavior of a cut end.

Fig. *L*.

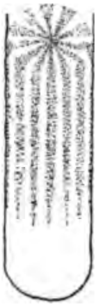


Fig. *M*.

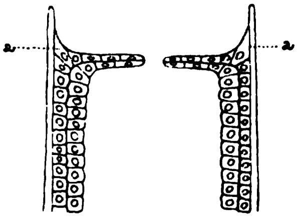


Fig. *L*. Closed end cut close to tentacle anlagen (edge of perisarc at distal end omitted for the sake of clearness).

Fig. *M*. Somewhat diagrammatical representation of a median longitudinal section of a closing end showing the closing membrane, composed of ectoderm and endoderm; and (*a*) ectoderm cells clinging to the perisarc.

In the course of the preceding experiments, several cases like that shown in Fig. *L* came under my observation. Here the cut had accidentally been made in the distal end of the proximal tentacle anlagen instead of in the space between the two anlagen as in Fig. *B*. As the figure shows, the edge was drawn in toward the center, the ends of the anlagen forming radiating sectors of the circular closed end. This seemed to me to show clearly that my previous observations were accurate, and that the cut edge does bend over and then move toward the center, stretching out the tissue and thus giving the radial appearance of the pigmented stripes, and of the ectoderm cells in MORGAN's silver nitrate preparations. It would be an interesting experiment to cut through the tentacle anlagen in various planes, and watch the closing in, with the stripes as landmarks.

One might illustrate this closing-in process as follows: Let a piece of very elastic rubber tubing, white on the outside and black within, represent the ectoderm and endoderm, or rather the coenosarc, of a piece of Tubularia stalk. Cement this within a piece of glass tubing which will represent the perisarc. Loosen the cut end of the rubber from the glass and apply sufficient centripetal force to several points at the edge to draw it to the center, pulling the rubber tubing from the glass, but with such difficulty that the rubber is sufficiently stretched to form a smooth membrane without gathers at the center. Now if the ectoderm cells or the pigmented stripes have been outlined in ink on the outside of the rubber tube, we have something similar to a closed end of Tubularia, — a membrane thinner than the rest of the coenosarc, with cells elongated radially; or, as in Fig. *L*, the ends of the tentacle anlagen forming sectors of the circular membrane.

My observations lead me to think that the force that closes a cut end of Tubularia acts centripetally in the cells at the cut edge.

Two hours or so after pieces are cut, the membrane usually presents a perfectly level surface (Fig. *H*), and a little later it begins to curve outward (Figs. *I* and *J*); still later, in very many cases, the coenosarc with the thin layer of perisarc which has formed over it, projects out beyond the perisarc (Fig. *K*): this is almost invariably the case in pieces where the distal anlagen have been cut off (Figs. *D* and *E*). The »thin end« described by MORGAN ('01) seems in such specimens to be formed wholly by extension of the coenosarc forward.

One is inclined to suspect that this thinning out of the distal end by extension forward, — or possibly in some cases by withdrawal of material into the tentacle region, — is a significant feature in the process of regeneration, and that it may be concerned in the respiration and nutrition of the developing hydranth.

The layer of perisarc which is formed over the closed end of the coenosarc in an early stage (Fig. *H*), appears to remain extensible; for, when the hydranth is emerging from the tube, it is often seen stretched out over the hydranth to a distance equal to three or four times the diameter of the tube: finally it breaks away around the edge of the tube and is thrown off by the expanding tentacles, as the calyx-cap of a poppy is thrown off by the expanding flower.

I take this opportunity to express my appreciation of the opportunity for research afforded to me both at the Marine Biological Laboratory, Woods Hole, Mass., and at the Zoological Station at Naples, through the kindness of the »Association for maintaining the American Woman's Table at the Zoological Station at Naples and for Promoting Scientific Research by Women«. I also wish to thank the professors and attendants at both stations for their unfailing kindness and zeal in procuring for me the material that I desired.

Conclusions.

1) There is no quantitative relation between the number of red pigment granules in the circulating fluid of regenerating pieces of Tubularia, and the amount of red pigment in the new hydranth. The red granules are thrown into the circulation by degeneration of endodermal ridges or tentacle anlagen: they usually accumulate in a mass, or ball, at the distal end of the tube and are ejected as waste material by the hydranth.

2) The force which closes the cut end of a piece of Tubularia stalk acts in the cells at the cut edge rather than in the whole material which forms the membrane. This is shown 1) by the appearance of the ectoderm cells at the periphery of the closing membrane, which is gradually pulled away from the perisarc by the centripetally acting force; 2) by the radial appearance of pigmented stripes, as in Fig. *L*; 3) by the radially elongated ectoderm cells of the membrane (MORGAN, '02).

Würzburg, Germany, June 1, 1902.

Zusammenfassung.

1) Es giebt keine quantitative Beziehung zwischen der Anzahl der rothen Pigmentgranula in der Cirkulationsflüssigkeit regenerirender Stücke von Tubularia und der Menge des rothen Pigments in dem neuen Hydrant. Die rothen Granula gelangen durch Degenerationsvorgänge der Führungsleisten oder Tentakelanlagen in die Cirkulation. Sie häufen sich gewöhnlich am distalen Ende der Röhre zu einem Klumpen oder Ball zusammen und werden von dem Hydranten als unnützes Material ausgeworfen.

2) Die Kraft, welche das Schnittende eines Stengelstücks von Tubularia schließt, hat ihren Sitz mehr in den Zellen am Schnittrande, als in dem ganzen Material, welches die Verschlussmembran bildet. Darauf weist hin 1) das Erscheinen der Ektodermzellen an der Peripherie der Verschlussmembran, indem sie schrittweise durch die centripetal wirkende Kraft vom Perisark abgedrängt werden, 2) durch das Auftreten radiär gestellter Pigmentstreifen (vgl. Fig. *L*); 3) durch die radiale Verlängerung der Ektodermzellen der Verschlussmembran (MORGAN, '02).

Literature.

DRIESCH, H., '99, Quantitative Regulation bei der Reparation der Tubularia. Archiv f. Entwickelungsmech. IX. 1899.

MORGAN, T. H., '01, Regeneration in Tubularia. Archiv f. Entwickelungsmech. XI. 2. 1901.

—— '02, Further Experiments in the Regeneration of Tubularia. Archiv f. Entwickelungsmech. 1902.

STEVENS, N. M., '01, Regeneration in Tubularia mesembryanthemum. Arch. f. Entwickelungsmech. XIII. 3. 1901.

The Relation Between Normal and Abnormal Development of the Embryo of the Frog, as Determined by Injury to the Yolk-Portion of the Egg.

By

T. H. Morgan.

With Plates VIII—XII and 3 figures in text.

Eingegangen am 10. Juni 1902.

Sep.-Abdr. aus Roux' Archiv für Entwickelungsmechanik, Band XV, Heft 2. 1902.

Table of Contents.

page
Introduction 239
Part I. Review and critique 241
  1. Relation of the cleavage-planes to the planes of the embryo 243
  2. Location of the first plane of cleavage 246
  3. Meridional position of the embryo 248
  4. Location of the dorsal lip 249
  5. Extent of overgrowth of the dorsal lip 250
  6. Ring embryos, and spina bifida 255
  7. Gastrulation 259
  8. Influence of external agents:
    a. Gravity 263
    b. Centrifugal force 264
    c. Salt solutions 266
    d. Oxygen 268
    e. Temperature 269
Part II. Experimental 271
  1. Abnormalities produced by centrifugalizing the eggs 271
  2. The immediate effect of the centrifugal force 288
  3. Later stages with yolk-plug 290
  4. Effect of cold on the development 291
  5. Ring-embryos 293
  6. Embryos with open medullary folds 294
General discussion of results 294
  1. Half-embryos 294
  2. Whole embryos along one side of the embryonic rim 295

page
3. Anterior embryos 298
4. Dorsal embryos 299
5. Ventral embryos 300
6. Pieces of embryos, and embryos of reduced size 300
7. Blastoporic rim 301
8. Anal invagination 302
9. Abnormal embryos of the frog in relation to the specific action of modifying influences 304
Summary 306
Zusammenfassung 308
Literature 309
Description of Figures 313

Introduction.

The following studies bear directly on the general problem as to how far, and in what sense, the results of abnormal development can be used to interpret normal development. An examination of the literature shows that two diametrically opposite views are held by embryologists at the present time. One school maintains that the evidence from abnormal development can be applied directly towards the elucidation of the normal development. The other school maintains that just so far as the embryo is abnormal it departs from the normal method of development, and the evidence from the one source has no application to the other.

It is our purpose to attempt to show on the evidence furnished by the abnormal types of development of the frog's egg that these two opinions rest, in part, on a misconception of the relation of the two processes; for, while on the one hand it is now clear that if the embryo is prevented by changed conditions from following its usual, normal course it may often reach its goal in an indirect way, yet, on the other hand, it is also possible that in doing so it may proceed along the same lines followed by the normal embryo, as far as the changed conditions allow.

The fundamental question is whether, when the normal course of development is changed the embryo re-forms independently of the old path already outlined in the organization, or whether it follows the old lines, except where prevented from doing so by some one or more of the new conditions imposed upon it. A specific example may make this clearer.

If the first plane of cleavage of the frog's egg corresponds more or less to the plane that divides symmetrically the dorsal lip of the blastopore, and if by artificial means the first two blastomeres are separated from each other, will the two new dorsal lips, one on each half-embryo, appear on the same part of the egg where the whole one would have appeared?

Or again. If after the second cleavage the egg is separated in two parts in the plane of this second cleavage, will the two anterior blastomeres make a new embryo with the old organisation as a basis? Will the two posterior blastomeres also re-organize with the new dorsal lip on that side that would have been nearest the whole dorsal lip? If the latter change should take place in this way we can see how futile are the two points of view mentioned above. If it be objected that in these examples there is no abnormal development at all, but the separated parts of the egg develop normally, I do not admit that the objection is valid. In order however that there may be no disagreement let us take another case where the abnormality will be admitted on all hands.

The abnormal form called spina bifida (or asyntaxia medullaris) has an exposure of yolk on what appears to be the dorsal side of the embryo. At the anterior end there may be a single medullary plate, or tube, which divides at the yolk-exposure into halves, each half of the normal single plate. One group of embryologists claim that this condition shows that the material to form the nervous system is laid down as a ring which normally closes over the yolk to form a single median structure, the medullary plate. This school claims that the condition of spina befida means that this same ring has been prevented from closing over the yolk, and has formed, in situ, its half-structure.

The other school claims that we can not conclude anything at all in regard to normal development from this abnormal kind of embryo.

Both points of view are, I believe, erroneous. The first because it can be demonstrated in some cases beyond reasonable doubt, that the whole, or a half of the medullary plate may be formed in a region of the egg where the material is not that which goes to form the same parts of the normal embryo. The second view is erroneous, because, wherever the embryo is formed the same general plan of development is followed.

The difference of opinion on this point has arisen, as I shall try to show, on a false view of what is to be compared, and the difficulty is not dissimilar form that into which comparative anatomists have fallen. If, for instance, we compare the series of appendages ot one animal having, let us say, six serially graded pairs with another animal having twelve similar pairs with a longer gradation between the first and the last, it would be manifestly absurd to compare directly any one of the first series with any one of the second, and yet it is obvious that both series admit of a sort of ideal comparison. The general method of formation is the same in both, and the structure is on the same plan, but we go wrong if we attempt to find out, which one of the first series corresponds to which one of the other. So it is, I believe, with the normal and abnormal types of development. We err when we try to show how the abnormal form is a part of the normal development, — it may be, but often it is not the same. Our only safe ground is to admit frankly that the plan in the one case is more or less the same plan or ideal followed in the other, but the material may not be the same material in the two cases. The location of the parts may be and often is altered, but the alteration is along the lines of the old organization, which gives the basis for the new form.

This leads to the following consideration. The embryo has great capacity to adapt itself to change, but if in doing so it moves along the lines of the old organization already laid down, our problem will be to determine what part of the old organization is made use of in the re-organization. The experimental part of this paper will deal directly with this problem.

Part I. Review and Critique.

The relation of the egg to the embryo that develops out of it is at the present time the central problem of embryology. This was not so much the case in the early days of embryology, during the 17th, 18th, and 19th centuries, when the idea of preformation lead to the conception of the egg as an envelope enclosing a miniature embryo, — as the leaf is enclosed in the bud, or as the skin of the chrysalis conceals the butterfly. For the preformationist there was no problem of importance connected with the position of the embryo in the egg, since it mattered little how the embryo lay within its envelopes, so long as its parts were in existence. When, however, the idea of epigenesis took shape the central and all important problem lay in the relation of the egg to the embryo. At first, when the egg was looked upon as a sphere of unformed material, as WOLFF had supposed, the problem of position, or localization did not occupy the fore-front of embryological speculation. As soon as it was recognized, on the other hand, that the egg itself had a definite structure, then the all-important problem for the embryologist became that of determining the relation between the egg-structure and that of the embryo; and since the most tangible problems are those connected with the localization of the parts of the embryo, the thoughts of embryologists turned in this direction. The field of investigation which has been most thoroughly explored at present is that of the development of the egg of the frog; and as the outcome of a large number of observations and experiments on this egg it may fairly be claimed that we have made in recent years no small advances. It may, therefore, not be unprofitable to examine the evidence that has lead to our present position.

The earlier attempts to localize the embryo in the egg were not very successful. The early students of the frog's egg, RUSCONI and VON BAER, for instance, thought that the embryo developed on the black hemisphere. The striking experiment of NEWPORT in 1851 did not attract the attention it deserved, and it was not until after the brillant achievements of PFLÜGER and of ROUX that the relation between the cleavage process and the formation of the embryo attracted widespread interest. In the same year, 1883, both PFLÜGER and ROUX determined once again, and without knowing of NEWPORT's results, that the first plane of cleavage of the frog's egg corresponds in position with the median plane of the body of the embryo. PFLÜGER's further experiments carried out at this time lead him to believe that the egg is isotropous, and subsequent investigation has, in a sense, confirmed this conclusion, provided by isotropy we mean that all parts of the egg have the power of producing any part of the embryo — an idea that is more accurately expressed at the present time by the word totipotence.

If, however, we mean by isotropy that the egg in homogeneous, the idea is probably false, since we are now in a position to maintain on good evidence, I believe, that a heterogeneous although labile organization must be postulated for the egg. The results of PFLÜGER's experiments point to a totipotence of the egg-material, but PFLÜGER's conception of isotropy appears to have been nearer to that of homogeneity, since he compares the cells to the flakes of snow that go to form a glacier.

ROUX, on the other hand, was lead by his experiments to a conception of development which is difficult to classify either under the term preformation or epigenesis, but contains, as he has insisted, elements of both doctrines. Nevertheless his mosaic theory of development, which is the most characteristic feature of his view, is one that supposes the egg to become during its cleavage a heterotropic structure; the machinery for development being given in the preformed elements of the nucleus, and in the mechanism for the separation of the elementary structures into their components. The protoplasm of the egg plays a secondary rôle so far as preformation is concerned, and is treated as a sort of indifferent medium that acts only as it is controlled by the elements of the nucleus. Its differentiation is consequently the result of a sort of epigenetic development. The protoplasm is, in fact, looked upon as a substance out of which any or all parts of the embryo can be formed under proper guidance. It is totipotent in a passive sense.

With this preliminary statement we may proceed to examine a large body of evidence on which our present opinion must rest. Roux found that the fertilized egg of Rana esculenta orientates itself with its dark pole to one side of an imaginary vertical line passing through the center of the egg. In consequence of this oblique position of the fertilization egg, a small portion of the white hemisphere can be seen when the egg is looked at from above. We shall speak of the white portion of the egg that appears above as the grey crescent. Roux found ('83) that when the first furrow comes into the egg it passes through the black pole, through the highest point of the egg, and the egg center. It lies, therefore, in that meridian of the egg that divides symmetrically the grey crescent. Roux also found that the second furrow, which is at right angles to the first, lies in the upper dark hemisphere nearer to the grey crescent, and that the two cells on the crescent side of the egg are somewhat smaller than those of the opposite side. His figures also show that after the third cleavage two of the upper cells are smaller than the opposite ones. It is important to draw attention to this last point, since it has, to some extent, been lost sight of by later writers, who have gone over the same ground. Roux supposed, at this time that the embryo is formed on the dark hemisphere, and since the medullary folds extend backwards to that side of the egg over which the grey crescent was seen, he located at first the posterior end of the embryo in this region, and the anterior end at the opposite side of the black hemisphere.

Pflüger showed that this orientation is wrong, and that the embryo appears in reality over the white hemisphere. A secondary rotation of the whole egg, just before the embryo appears, brings the embryo to the top of the egg. The head of the embryo, and not the tail, develops in the region of the white crescent.

Roux observed that there is very often a discrepancy between the position of the first plane of cleavage and the median plane of the embryo. He attributed this to accidental shifting in position of the egg during development, and since he did not compare the position of the two planes in question until after the medullary plate had appeared, there can not be much doubt that such shifting might take place. The results might have been more accurate had the first appearance of the dorsal lip of the blastopore been taken as indicating the future mid-dorsal line of the embryo, for, by doing so, the possible error of a shifting of the egg on its axis during the overgrowth of the blastopore is avoided, and also the much greater likelihood of shifting as the whole egg rotates to bring the embryo uppermost. It is very doubtful, however, in the light of more recent work, whether even with these precautions there would have been found a constant agreement between the first plane of cleavage and the median plane of the embryo.

In later papers Roux advanced the idea that the second plane of cleavage divides the material for the anterior half of the embryo from that of the posterior half; but this question can be best considered after we have discussed the meridional position of the embryo on the egg.

As has been stated, Roux found that the axis of the fertilized egg of Rana esculenta is inclined at a considerable angle. He found on the other hand that no such declination is present in the egg of Rana fusca; but Schultze observed, on the contrary, in the latter egg after fertilization a very marked declination of the axis. The important question then arose as to whether a declination of the primary axis will appear in the unfertilized egg if it is thrown into water, and if it does whether it corresponds to that of the fertilized egg. Roux tested this point indirectly by the ingenious experiment of localized fertilization. An egg was taken from the uterus, placed between two glass plates and enough water added to allow the jelly-membrane to swell somewhat. After thirty minutes a solution containing spermatozoa was brought in contact with one side of the egg. Later (after ten minutes) more water was added, when the membranes swelled further so that the egg became free to rotate. When the first furrow appeared it was found to pass, more or less, through the region at which the spermatozoa had been set free, and where presumably one had entered the egg. Roux found that the egg rotates after fertilization on its axis, so that the black pole turns towards the side at which the spermatozoon is supposed to have entered. In consequence of this rotation the grey crescent appeared at the side exactly opposite to that of entrance of the spermatozoon. The first furrow passed through the fertilization point and the black pole and, therefore, divides the grey crescent symmetrically. Hence since the spermatozoon could be made to enter at any point of the upper hemisphere, and since this determines the position of the first plane of cleavage and the median plane of the embryo, it appears to follow that before fertilization all primary meridians of the egg are equipotent[1]).

These results might seem to furnish a demonstration of the equivalency of all primary meridians, but serious doubts have been raised as to the certainty of this conclusion.

In the first place, Roux himself had observed that unfertilized eggs of Rana esculenta slowly rotate, so that the black pole becomes declined. Does this mean that a pre-existing plane of organization exists in the egg? If so, does it have the same symmetrical relations as that of the fertilized egg? Does normal fertilization take place in a predetermined region, and if so does this region lie opposite the region that becomes the grey crescent? Is it not possible that the eggs in Roux's experiment were under abnormal conditions, so that in them an unusual point of entrance of the spermatozoon was forced upon the egg? If so the results can not be compared with what takes place in the normal egg.

The only ground at present on which we may attack Roux's conclusion is based on certain observed relations pre-existing in the egg before fertilization. Schultze, in particular, has attempted to throw doubt on Roux's results. Schultze has shown ('87) that ripe ovarian eggs of several amphibia show, before they have entered the oviducts, the germinal vesicle in an excentric position in relation to the black hemisphere, and, in consequence, there is a bilateral plane present in the egg. The polar spindle also lies in the same excentric position and it could be shown that the first plane of division often passed through the point of extrusion of the polar bodies. This makes it probable, SCHULTZE thinks, that the early bilaterality of the egg coincides with the future plane of bilaterality of the embryo, although he admits as possible that a change might occur so that a new plane of bilaterality is determined after fertilization; but he thinks this very improbable. SCHULTZE does not fail to point out certain inconsistencies in ROUX's results. For instance, ROUX made his statement only in regard to Rana fusca, and not for Rana esculenta, because in the former he found that the primary axis is vertical, and hence any possible plane may become the plane of first cleavage. ROUX thought that in Rana esculenta, owing to the obliquity of the axis of the fertilized egg, the entering spermatozoon might be carried secondarily by currents into the symmetrical plane of the egg. SCHULTZE finds, however, that the unfertilized egg of Rana fusca has also an oblique axis, and often, very often, one that is markedly oblique. If so, ROUX's argument should also apply to the latter species. SCHULTZE sums up his conclusion in the non-commital statement, that at present it has not been shown that under normal conditions there is no preformed region at the which the spermatozoon enters. He claims that ROUX's experiment shows, at most, that in eggs held artificially in a given position the spermatozoon may enter at some other point than that at which it would normally enter, and in doing so determines the plane of first cleavage. If the last argument be admitted we have reached at least a formal agreement between the two apparently opposite results. I should also like to add here that even if the position of the first plane of cleavage may be determined by the artificial, localized fertilization, it may still be possible that the location of the embryo on the egg may be predetermined by other internal factors. The median plane of the latter by no means always corresponds to the position of the first plane of cleavage. Let us consider this point somewhat further.

---

[1]) I purposely avoid the use of the word totipotence here since the meridians might be equipotent without being totipotent.

As has been stated, NEWPORT, PFLÜGER, and ROUX found that, in general, there is an agreement, more or less exact, between the position of these two planes, but later writers have by no means given unanimous consent to this conclusion. In fact, NEWPORT himself observed that »at times the axis deviates to the right or left« and, as previously stated, ROUX's and PFLÜGER's observations showed often a lack of coincidence in the two planes. Even more suggestive is **Pflüger**'s discovery that while the planes of cleavage vary greatly under certain conditions, the position of the embryo on the egg does not follow the changes of cleavage planes, but belongs rather to one of the primary meridians of the egg. **Rauber**'s observations ('86) showed also that a difference often exists between the position of the first cleavage plane and the median plane of the embryo. He severely criticises **Roux**'s hypothesis, and tries to show for the frog, that the second plane of cleavage more often coincides with the median plane of the embryo. **Morgan** found ('91) a general agreement between the first plane of cleavage and the median plane of the embryo in the frog, Chorophilus triseriatus, but states that the two do not always exactly coincide. **Jordan** and **Eycleshymer** ('94) pointed out that during cleavage the planes so shift from their first position, that cells derived from the right blastomere are often carried over to the other side, and vice versa.

Schultze also ('90) found that the two planes in question often coincide. **Roux**, in 1892, made the significant admission that in bilaterally symmetrical animals one or the other of the first two planes of cleavage becomes the median plane of the body, and since in his experiments on the frog's egg he sometimes found the median plane of the embryo coinciding with the second plane of cleavage, he thought that the two planes could be interchanged as a result of some sort of »anachronism« of the cleavage.

Hertwig in 1893 criticised severely **Roux**'s conclusion, and stated that while in many instances the planes of cleavage coincide with the planes of the embryo, yet there is no causal dependence of the two phenomena on each other, but both are connected with the »organization of the egg«.

Eycleshymer ('95) concluded that in the frog, and in some other vertebrates »no cleavage furrow .... bears a fixed relation to the future median plane of the embryo«. **Kopsch** ('95) found, as had **Born** ('93) and **Hertwig** ('93), that in eggs held between two parallel compressing plates there is no absolute relation between the first furrow and the median plane of the embryo, although **Roux** claimed ('94) that the two planes coincided in 80 % of cases. In the same year **Roux** objected to his conclusions from compressed eggs being extended to the normal egg.

Kopsch ('95) made photographs of eggs under normal conditions, and found that even with the greatest precautions the eggs shift their position during cleavage. In 1900 he gave a detailled account of these experiments, and concluded that Roux's methods could not have given exact results. He agrees with the view that the shifting of the blastomeres during cleavage makes it impossible that an exact coincidence between the two planes could exist, but admits that »within certain limits (not yet definitely determined) a certain relation is found between the first plane of cleavage, the median plane of the embryo, and the organisation of the egg as shown by the work of Roux, Born, Hertwig, O. Schultze, Morgan and Ume Tsuda, and others with which my own experience agrees«.

This review of the literature shows that while the first plane of cleavage of the frog's egg¹) often coincides more or less with the median plane of the body, yet the two do not invariably do so, and since normal embryos arise under all degrees of divergence the importance of the Newport-Pflüger-Roux discovery seems not to be so great as was once supposed. That there is any necessary connection between the two planes under discussion, in the sense that the position of the one is dependent upon that of the other, seems certainly disproven. This conclusion can be most easily demonstrated by altering the sequences of the cleavage planes by compressing the egg. Pflüger first instituted this method of experiment, and it has been repeated by Roux, Born, Schultze, and others. The main result is that by compressing the egg so that its form is changed the position of the cleavage planes is different from the normal, and, in consequence, the distribution of the nuclei, so far as the sequence of their formation is concerned, is different from that of the normal egg. Nevertheless, a normal embryo develops. It has not, unfortunately, been definitely ascertained whether the embryo appears in the same position on the compressed egg as on the normal egg.

A wide divergence of opinion also exists as to the meriodional position of the embryo on the egg. Pplüger and Roux (in later papers) were convinced that the embryo lies over the lower hemisphere of the egg, and not on the black hemisphere as had been previously supposed. The material from which the embryo forms is not, however, that of the white cells of the lower hemisphere, but

---

¹) I have intentionally omitted references to the urodele amphibia. In the axolotl Rauber ('86) believed the second plane of cleavage to coincide with the median plane, and Hertwig obtained the same result for Triton, Jordan ('94) for the newt, and v. Ebner for Triton. Clapp ('91) determined in the teleost, Batrachus, and Morgan ('93) in Ctenolabrus, that there is no agreement at all between the planes of cleavage and embryonic axes.

it is formed from the darker cells that grow over the lower hemisphere during the process of gastrulation. It was pointed out by MORGAN ('91) and later by MORGAN and TSUDA ('93) that during the later cleavage-period the cells in the upper parts of the white hemisphere become more deeply pigmented, and that there is a progressive splitting off of small black cells which are added to those already present in the upper hemisphere¹). These new cells form a part of the ectoderm of the embryo. As a result of this change, the egg is more pigmented superficially at the time of gastrulation than when cleavage first began. Consequently when the dorsal lip appears below the equator of the egg it stands just below²) the advancing edge of pigment on that side of the egg on which the grey crescent was present. In regard to the last point ROUX, SCHULTZE, MORGAN and TSUDA, and H. V. WILSON are in complete agreement. It is not improbable, however, that the coincidence between the position of the middle point of the grey crescent and of the blastopore is only approximate, as shown by MORGAN and TSUDA³). The author has also within a year determined beyond a question that in Rana palustris the blastopore appears on the crescent-side of the egg.

SCHULTZE has stoutly maintained the older view, viz., that the embryo is formed on the dark hemisphere, while other writers have tried to show that the embryo develops, in part, out of the dark hemisphere, and in part over the white hemisphere. In order to examine the evidence advanced for these different opinions it will be necessary to enter somewhat more fully into the process of gastrulation of the frog's egg.

On that side of the egg where the grey crescent appears the cells are smaller than on the opposite side at a corresponding distance below the highest point of the egg. ROUX was, I believe, the first to make this observation. Even after the second cleavage he observed that the two cells on the crescent side were smaller than

---

¹) H. V. WILSON ('02) has confirmed this result.

²) The relation of the dorsal lip to the edge of pigment is undoubtedly different in different forms, although it seems probable that the blastopore appears on the same side in all cases.

³) H. V. WILSON has followed EYCLESHYMER in ascribing to MORGAN the opposite point of view from that which he really held, due probably to a misunderstanding of what was meant by the dark and light sides of the egg. In reality both WILSON and EYCLESHYMER confirm the view held by ROUX, SCHULTZE, and MORGAN. KOPSCH also came to the same conclusion.

those of the opposite side. SCHULTZE also discribed in '87 the smaller cells on the lighter side of the egg of Rana fusca. MORGAN and TSUDA have found a somewhat different relation to exist in the egg of Rana sylvatica and Rana palustris. They found that, as a rule, one of the upper four cells of the eight-cell stage is smaller than any of the others. Its vis-a-vis is generally the largest. The smallest cell lies nearest the top of the grey crescent. Sometimes, however, two of the cells on the crescent side are smaller than the opposite two. The later cells on this side of the egg are also smaller than those on the opposite side. At the four-cell stage no differences in the size of the cells can be observed, as ROUX maintained, except sometimes in eggs that depart markedly from the general type. Not only, according to MORGAN and TSUDA, does the first cleavage often fail to divide symmetrically the grey crescent, but the position of the smaller cells at the eight-cell stage also varies, as stated above, in relation to the crescent.

In general, most authors are agreed that the dorsal lip of the blastopore appears beneath the equator of the egg, and on that side of the egg, as has just been stated, on which the grey crescent was seen at first. On the opposite side of the egg, owing in part to the obliquity of the egg axis, the pigment extends somewhat further down towards the lowest point of the egg. In regard to the exact distance below the equator at which the dorsal lip of the blastopore appears there may be differences in different species. The lip has been variously located at or near the equator, to as far down as 45 degrees below that level.

We come now to the much disputed question of the changes that take place during the formation of the blastopore and during its closure. ROUX and PFLÜGER supposed that the dorsal lip of the blastopore advances nearly 180 degrees over the lower hemisphere, and that the embryo (as judged by the length of the dorsal nerve plate) extends for a similar length over the lower hemisphere. SCHULTZE has contended repeatedly that the dorsal lip remains stationary, and that the embryo is formed in front of it on the black hemisphere. The apparent advance of the dorsal lip as it approaches the lowest part of the egg during gastrulation is due, he believes, to a rotation of the whole egg, which is the result of a change of its center of gravity during the formation of the archenteron.

A number of observers all agree that SCHULTZE is wrong in his contention. They agree that an actual advance of the dorsal lip takes place, but they disagree amongst themselves as to the extent of the overgrowth. The evidence on which their conclusions rest is derived from several sources, and needs careful consideration. We may examine this evidence under the following headings; 1) Results from sticking various regions of the egg; 2) Results from holding the egg in a fixed position between glass-plates, or by preventing the membranes from swelling; 3) Results from certain abnormal embryos showing Asyntaxia medullaris (or Spina bifida, HERTWIG).

1) ROUX first tried the experiment of sticking eggs in various regions in order to see if the resulting embryos would show defects corresponding to the region injured. If HIS' principle of localized germ-forming areas (»organbildenden Keimbezirke«) is correct, the loss of a part of the egg might lead to localized defects in the embryo, — provided regenerative changes did not take place. ROUX's first results were far from satisfactory, for the following reasons. First, because large injuries were made which, no doubt, would make the development abnormal to such an extent that it would be unsafe to draw conclusions from the results; 2) because many of the injuries were made during the early segmentation stages, so that a long time elapsed before the embryo was formed, and during this time »self-regulative« changes might take place; and, 3) because it seems not improbable that some of the results were due to a shifting of the extra-ovate. ROUX's critics have not been slow in pointing out these possible sources of error. ROUX's later experiments are not, however, open to the same extent to these criticisms, and strongly support his contention that the embryo develops over the lower hemisphere.

SCHULTZE, in '89, carried out a series of experiments of somewhat the same kind. Quite small injuries were made with a needle, or by the application of a small drop of acid. In other cases natural markings of the egg were used as orienting points. SCHULTZE's conclusions based on these experiments are the opposite of those of ROUX, and he tries to show once more that the embryo lies on the black hemisphere. Amongst other results SCHULTZE's observations show definitely that the dorsal lip of the blastopore approaches any marks that may be present on the lower hemisphere. He explains this as due to the forward movements of the yolk cells towards the dorsal lip as the process of gastrulation takes place¹). SCHULTZE

---

¹) KOPSCH's photographs show that such a movement does actually take place.

explained the sinking down of the dorsal lip, that takes place during this time, as the result of a rotation of the whole egg in this direction. He found that marks on the black hemisphere of the egg hold approximately their position in respect to the blastopore; and from this he infers that the dorsal lip is, morphologically, a fixed point. SCHULTZE's argument appears faultless, but is not, I believe, beyond criticism. He admits an advance of the dorsal lip, and tries to account for it by a rotation of the whole egg, but he fails to show, I think, from the evidence of the marks that the whole egg does rotate. Several of his observations, in which the markings were in the upper hemisphere, show that the dorsal lip increases its distance from them as development proceeds. This can not be explained on his view. He leaves out of account the behavior of the ventral lip of the blastopore which appears about 100 degrees from the dorsal lip. Since the dorsal and ventral lips ultimately come very near together we must suppose, on SCHULTZE's view, that the ventral lip advances, or is carried over 100 degrees. There is no evidence forthcoming to show that such an advance takes place, and much evidence to show that the actual advance is very much less than this amount.

MORGAN and TSUDA carried out a number of sticking experiments and arrived at a conclusion which in the main supported ROUX's and PFLÜGER's view, viz., that the embryo forms over the lower hemisphere. They thought that only the anterior end of the head is formed from the region in front of the dorsal lip. In one important point they differed from the ROUX-PFLÜGER view. They found that the entire length of the embryo, when it first appears, is only 120 degrees of the periphery of the egg, hence since the anterior end of the embryo lies in front of the first position of the dorsal lip the advance of the latter must be less than 120 degrees, and probably not more than 100 or 110 degrees. This conclusion also agrees with the actual results of their observations as to the extent of the advance of the dorsal lip. If, in fact, the dorsal lip advances over 180 degrees, as claimed by ROUX, it would cover a region greater than that on which the embryo is at first formed, and this stands in contradiction to ROUX's statement that the anterior end of the embryo lies just in front of the region at which the dorsal lip appears. Later writers have also confirmed the view that the advance of the dorsal lip is less than that supposed by ROUX. We may consider, in sequence, the views of other workers, that bear on this point.

In a paper in '94 MORGAN gave some further observations supporting his earlier view. A new series of experiments were carried out with the purpose of determining, whether the material of the dorsal lip advances backwards along the sides to produce the lateral lips of the blastopore, or whether the latter are formed in situ. The results showed that the latter alternative is true. The experiment also has a bearing on the production of embryos with asyntaxia medullaris, since it makes probable the view that these embryos are not formed by a backward migration of the material of the dorsal lip along the sides, but that they develop from material already in position to form the sides of the embryo. This view indicates that SCHULTZE's contention, that the embryos with asyntaxia medullaris are formed by a process totally different from the normal process of development, is without foundation.

ASSHETON carried out some sticking experiments in '95 and concluded from them that the dorsal lip advances not more than 60 to 70 degrees, and that quite a large part of the anterior end of the embryo forms anterior to the first position of the dorsal lip. His figures indicate that this region is about 60 degrees in length, or half the length of the early embryo.

In the same year EYCLESHYMER published the results of a number of experiments of this same kind. His results, obtained by injuring the egg at the four-cell stage, and during the early cleavage, are quite inconstant. He concludes from his experiments, nevertheless, that the greater portion of the embryo (Acris grillus and Bufo lentiginosus) lies anterior to the first position of the blastopore, and extends to the black pole of the egg. KING (see infra) who has recently carried out a large number of experiments on Bufo gives strong reasons for dissenting from EYCLESHYMER's conclusions.

BERTACCHINI ('99) also made numerous sticking experiments, and concluded from them that the dorsal lip moves over nearly 180 degrees.

H. V. WILSON ('00) carried out some sticking experiments on the eggs of Chorophilus feriarum. He also made a number of observations on the movements of marked cells. The yolk-cells, adjacent to the dorsal and ventral lips, were observed to disappear under the lips. Ectoderm cells close to the dorsal, or to the ventral lips were seen to disappear around the edge of the lip as they were rolled under. WILSON found that the dorsal lip appears 15 degrees below the equator of the egg, and travels 25 degrees over the yolk before the ventral lip appears. The distance at this time between the two lips is about 95 degrees, which is subsequently covered, in part, by the further backgrowth of the dorsal lip, and, in part, by the forward growth of the ventral lip. If the rate of advance of the two lips is the same, as the author believes, and as MORGAN had also described, the dorsal lip must travel in all $25 + \frac{1}{2} \cdot 95 = 72$ degrees. Since WILSON thinks that the young embryo covers at first 120 degrees this will leave 48 degrees for the part of the embryo that develops in front of the first position of the dorsal lip. This means that somewhat more than one-third of the embryo is formed in front, and about two thirds by the backgrowth of the dorsal lip. The anterior end of the embryo will extend 33 degrees above the equator of the egg, and not 95 degrees as claimed by EYCLESHYMER. There is one point in these results that appears to me open to criticism, viz., that insufficient allowance is made for the thickness, or depth of the inturned edge of the ventral lip, and, therefore, too great a distance is assigned to the advance of the ventral lip. If we allow one-third of the supposed advance of $47\frac{1}{2}$ degrees of the ventral lip for the depth of the wall, it leaves about 16 degrees to be added to the advance of the dorsal lip, making the latter advance $25 + 47\frac{1}{2} + 16$ degrees, or 88 degrees. My own opinion is that this is near the actual distance travelled by the dorsal lip of the embryo of some species at least, and may even fall short of the real distance which appears to be somewhere near 100 degrees, or a little less.

KING ('02), who has quite recently made sticking experiments on the toad's egg, finds that the anterior end of the embryo is formed in front of the first position of the dorsal lip, and that the advance of the dorsal lip is about 120 to 140 degrees. The embryo when first formed being longer than that of the frog, and covering about 180 degrees of the circumference of the egg.

In conclusion it may be stated that while the results of these different writers are so much at variance as to indicate that the sticking method can not be relied upon as entirely accurate, yet, they show that if the method is used with caution it may furnish additional data for determining the location of the frog's embryo. It will be observed that all the later writers, with the exception of BERTACCHINI (whose results do not appear to me convincing) make the distance travelled by the dorsal lip much less than the 180 degrees postulated by ROUX's hypothesis. The results also show, beyond much doubt, that the posterior part of the embryo is formed by the backward growth of the dorsal lip. How much of the embryo is formed in front of the first position of the dorsal lip is still a matter of dispute, and seems to differ, to some extent, in different species. The evidence at hand makes it improbable that the embryo extends to the black pole, or even to the top of the egg, and it seems quite certain that SCHULTZE is wrong in his view that the whole embryo lies on the black hemisphere.

2) By adding only enough water to allow the egg-membranes to partially swell the egg may be held, in some cases, in any desired position, as first shown by PFLÜGER. Both PFLÜGER and ROUX have made use of this method to determine the position of the embryo on the egg. The method has also been used in connection with compressing glass plates. SCHULTZE has put in a disclaimer against the evidence derived from this source. He first urged that the condition being abnormal, we can draw no inference from the result as to what happens in normal development, but this really begs the question. Nevertheless there can be no doubt that the method can only be employed with great caution. Later ('80) SCHULTZE tried to show that, under the condition of this experiment, the egg, if turned into an abnormal position, either rotates back into a normal position, in which case it continues to develop normally, or else, if it remains in an abnormal position, it dies. These experiments were carried out, it is true, rather to determine the influence of gravity on development than to show the inaccuracy of the method as applied by ROUX, BORN, HERTWIG and others, but if SCHULTZE is correct his view renders invalid the evidence in regard to the position of the embryo on the egg. Since it has been clearly shown by ROUX ('00) and more recently by KATHARINER, that SCHULTZE's main contention in regard to the necessity of gravity as a factor of development is wrong, we can perhaps also dismiss SCHULTZE's other contention in regard to the location of the embryo. It must be admitted, however, that SCHULTZE's careful observations show how difficult it is to keep eggs that are developing fixed in an abnormal position[1]. He has undoubtedly pointed out a weak spot in the experimental evidence from this source.

3) Abnormal embryos of the frog are sometimes found in which the yolk-mass of the lower hemisphere lies exposed, to a greater or less extent, along the mid-dorsal line of the embryo between the halves of the medullary folds that extend around the exposed yolk.

---

[1] Except when the egg is placed obliquely at an early stage when a rotation of the contents takes place as shown by BORN ('83). Such eggs are, of course, worthless for determining the normal position of the embryo.

Roux was the first to describe embryos of this kind, and drew attention to their importance in throwing light upon the normal development. He describes these abnormalities as embryos having the condition of spina bifida. They were found more often in bunches of eggs laid towards the end of the breeding season.

Morgan ('93) produced these embryos artificially by adding 0,6 per cent. of sodium chloride to the water in which the eggs were developing. Bataillon ('01) has also described embryos of this sort from eggs placed in salt-solutions.

Many similar forms will be described in the second part of this paper. They were also produced by artificial means, viz. by subjecting the eggs to a centrifugal force under conditions somewhat different from those employed by Hertwig ('98).

In some of the spina bifida embryos the dorsal exposure of yolk is confined to a relatively small extent of the dorsal surface, while in others the embryos appear to be split open along the mid-dorsal line from the anterior connective of the brain to the anus at the posterior end, as shown in Fig. 53. An embryo of this sort extends as a ring around the equator of the egg, or just below the equator in some cases. Each half, therefore, covers nearly 180 degrees of the circumference. I shall speak of them as ring-embryos.

Roux concluded that these embryos arise from the same material as does the normal embryo, and that this material is in the form of a ring around the equator of the egg, and is 180 degrees in length on each side. He supposed that this material advances over the white hemisphere during the period of overgrowth of the blastopore, and the two halves uniting, fuse to give rise to the material of the dorsal side of the embryo. If, however, the early embryo only extends over 120 degrees, as certain observations referred to above seem to show, how can this fact be brought into harmony with Roux's view? We might assume that while at first the material to form the embryo extends over 180 degrees it draws together as it grows over the lower hemisphere until it covers not more than 120 degrees of the surface. On the other hand we might assume that the spina bifida embryo is formed out of material higher up on the egg than is that from which the normal embryo is produced. Or again we might attempt to make the results consistent by taking into account the advance of the lower lip. If, for example, it could be shown that the latter advances 60 degrees (or even something less) the harmony of the two results might be established. This would only be the case, however, on the supposition that by the length of the embryo we mean both the region in front of the point of final closure of the blastopore as well as that behind the latter. Let us consider these possibilities in turn.

1) There is no evidence showing that during the overgrowth there is a concentration in length of the material that forms the dorsal side of the embryo, and there is a good deal of evidence showing that a part of the anterior end of the embryo lies in front of the first position of the blastopore. The extent of this pre-blastoporic region is differently estimated by various writers. If any considerable amount of the anterior end lies in the pre-blastoporic region, then, since it can be shown beyond question that the distance between the points of formation of the dorsal and the ventral lips is over a hundred degrees (and probably as much as 120 degrees), it follows that the embryonic material must really shorten[1]) since its length is only 120 degrees when the embryo first appears. If this is not granted it follows that we can not extend the evidence from the spina bifida embryos towards explaining the length of the normal embryo. That this is really the case will be shown when these spina bifida embryos are further examined.

2) It can be shown, I think, that the spina bifida embryos sometimes form so high up on the egg that it is quite impossible that they are produced out of the same material that goes to make the normal embryo. This point was urged by MORGAN in 1894, when he tried to show that ROUX's conclusion in regard to these embryos is erroneous. Not that some of the material for the normal embryo may not be laid down as a ring, but that the material is from different parts of the egg in the two cases. A careful examination of the eggs from which the spina bifida embryos develop shows that the delamination of cells below the equator is retarded, so that at the time when the ring embryo is formed, the black-white edge is higher up than in the normal egg. The lips of the blastopore extend along this edge, as shown in Fig. 53 and above the lips the material to produce the halves of the embryo begins to differentiate. This accounts for the great length of the ring-embryos when the lips of the blastopore encircle the equator of the egg. An embryo of this sort is only ideally comparable with the normal, in the sense that in both cases some of the material appears in the form of a ring, but it is quite different

---

[1]) Even after the greatest possible deduction for the advance of the posterior lip has been made.

material in the two cases that produces the embryo. There is no preformed material that is common to the two kinds of embryos; there is only an ideal scheme of development followed out in somewhat the same way in both cases.

The point of special importance is that the position of the dorsal lip itself may be in some cases higher up on the abnormal egg than on the normal, hence we can not infer from these spina bifida embryos how much of the embryo of the normal egg lies in front of the first position of the blastopore. However, it is not improbable even if the blastopore first appeared in its normal position that if the lateral edge of the blastopore formed abnormally high up on the egg less of the anterior end of the embryo might appear in front of the blastopore than it does in the normal egg. This latter point is, however, largely hypothetical.

3) In regard to the advance of the ventral lip it must be admitted that in the spina bifida embryos this also lies open and its sides add themselves to the rest of the ring and make the embryonic region longer. If the ventral lip advances to the extent claimed by H. V. Wilson, viz., $47^1/_2$ degrees this would make the discrepancy between the length of the spina bifida and the normal embryo less than appears at first sight; for, if we admit that the dorsal lip travels over 120 degrees, and add to this $47^1/_2$ degrees, this amounts to $167^1/_2$ degrees, or roundly 170 degrees. It must be pointed out, however, that Wilson himself does not think that the dorsal lip covers as much as 120 degrees, and it seems to me improbable that the ventral lip advances as much as Wilson supposes, so that I can not accept this solution of the difficulty, but believe, as stated in the last section (2), that the extreme forms of the spina bifida embryos lie higher up on the egg than does the material of the normal embryo.

In further support of my view I should like to call attention to the following facts. In one of Morgan's ('94) figures (Plate 24, Fig. XVIII) the ring embryo lies above the equator of the egg, and the anterior end of the embryo is already nearly 45 degrees in length. If the posterior end of the open ring represents the posterior end of the embryo the latter would represent something like 45 + 190 degrees, or 235 degrees of the surface. This is, of course, an absurd estimate and shows, as I pointed out, that the evidence from these embryos can not be strictly applied to an interpretation of the normal development.

We can, of course, deny that in this case the posterior end of the open ring represents the posterior end of the embryo, but a comparison ot this with other similar embryos leaves no opportunity of escape in this direction.

In another figure given by Morgan ('94) (Plate 24, Fig. XIX), an embryo is drawn in which the anterior part extends over at least 100 degrees; yet as shown in this, and also in the following Fig. XX, the blastopore is open over an area measuring at least 95 degrees; and in addition a part of the posterior end is also formed. In this case it may fairly be claimed that the anterior end has begun to elongate, so that it is longer than when first laid down, but since the egg is still round it seems to me that this argument does not have much weight, and, in any case, the same interpretation can not be applied to the other embryo mentioned above.

In conclusion we may sum up the evidence in this section in the statement, that while Roux, Morgan and Hertwig appear to be justified in assuming that the evidence from these embryos indicates with some probability that the normal embryo develops in part as a ring which closes over the lower hemisphere, yet it is going too far to claim that the abnormal embryos show that the normal embryo develops from the same material that lies at the equator of the egg. I do not by any means agrees with Schultze in his belief that the evidence from these embryos is worthless as bearing on the normal development. On the contrary the evidence is of value, but only of relative value, and is to the used with caution. Taken by itself it may be inconclusive, but taken in connection with the evidence from other sources it helps to make probable the view that the normal embryo forms in part as a ring which closes over the lower hemisphere. At least a single hypothesis explains both the ring-embryos and the normal embryos, and in this respect the view has a distinct advantage over that of Schultze in which these ring-embryos are supposed to be something unique, whose development can not be brought into harmony with the normal development.

Gastrulation.

No less fertile in bringing forth a diversity of opinions is the question of the gastrulation of the amphibian egg. Since this topic lies somewhat outside of the range of our present examination it can be dealt with summarily. There are, however, for us one or two important questions. It will be admitted without discussion that what remains of the white hemisphere (after delamination as far as the edge of the blastopore) is covered over by the advance of the blastopore lips. SCHULTZE supposes that the yolk moves forward under the dorsal lip (which remains stationary), and that the ventral and lateral lips approach the dorsal lip[1]). Most other writers are agreed that the disappearance of the yolk is brought about by an advance of the rim of the blastopore, although some writers admit that at the same time there is a movement of the yolk-mass itself under the lips. In fact, that some such change as this takes place can be shown by the relation subsisting between the advancing margin of the blastopore and the yolk-mass. Curiously enough this point has scarcely been referred to by those who have tried to explain the process of gastrulation. Sections of the egg show that the lips of the blastopore are relatively thick structures, and if they grew over the surface of the lower hemisphere — the latter being stationary — the egg would have a much greater diameter at the end of gastrulation than before. I have made measurements of the living egg before and after gastrulation and find that the diameter is about the same. It follows, that the yolk must be drawn into the interior of the egg as the blastoporic lips advance, and this withdrawal is, to judge from appearances, the result of the activity of the yolk itself. It is this movement which is, in all probability, indicated by KOPSCH's photographs; and the evidence from the latter source can not, if this is the case, be brought forward to show the migration forward (or backward) of embryonic material, except, in so far, as this is due to a withdrawal inwards as the blastopore advances.

Any interference with the withdrawal of the yolk-mass will interfere with the overgrowth of the blastoporic lips, and if the lips of the blastopore represent the sides, or part of the sides of the embryo, a ring-embryo will result. This view will explain how we get the same kinds of abnormalities in so many different ways, such as by lack of oxygen, by the addition of salts or sugar to the water, by injuring the lower hemisphere, by the action of a centrifugal force, etc. The action of these different agents prevents the withdrawal of the yolk-cells, and, in consequence, the overgrowth of the blastoporic lips is interferred with. This explanation applies to all cases and the advantage of a single interpretation of so many different conditions will, I think, be generally admitted.

---

[1]) At least this seems the only possible interpretation on SCHULTZE's view.

During the period of withdrawal of the yolk-mass the segmentation cavity becomes smaller, and there can be little doubt that the yolk-mass moves into this space. Wilson especially has called attention to the ingrowth of a tongue of cells into the segmentation cavity in front of the anterior end of the archenteron. This advance may be interpreted, as Wilson appears to interpret it, as a forward growth of the archenteron. On the other hand it may be interpreted more simply as due to the yolk, that is drawn in, pushing forward more rapidly at the anterior end — in fact since the dorsal lip first appears at this end we should expect a more active withdrawal of yolk in this part to make way for the advance of the blastoporic lip. Whether this involves a forward extension of the cavity of the archenteron remains still to be determined. There is another possibility which no one has, as yet, taken into account, viz., that as the tongue of cells extends forward the ventral yolk-cells forming the anterior floor of the archenteron may roll around the anterior end of the archenteric cavity, and come to lie on the dorsal and dorso-lateral walls. Without more data it would be useless to discuss the possibility of this interpretation.

When the blastopore-lips have nearly overgrown the lower hemisphere, the archenteron is present as a narrow space beneath the embryonic region. It soon begins to enlarge and at this time the yolk-mass pressing into the segmentation cavity nearly obliterates it; and probably in consequence of this change the egg rotates as a whole so that the embryonic region comes to the top. The floor of the archenteron is formed of the yolk-cells which were at first the surface cells of the white hemisphere. A part of the anterior floor is also produced by the early drawing-in of cells at the dorsal lip of the blastopore¹). According to Assheton and others there is a splitting between the yolk cells in this anterior region, as a result of which the archenteric cavity is extended forward, but I do not think this view has been established.

The roof of the archenteron is formed by those cells that have been turned under around the dorsal and lateral lips of the blastopore. The anterior roof is formed by the first cells that are rolled

---

¹) A similar change takes place at the lateral and posterior lips of the blastopore producing the cells that form the edge of the floor in these regions.

under the dorsal lip of blastopore[1]. As the lateral lips come up to the middle line the cells that have rolled under their edge are added to those already in the mid-dorsal line. If we call the cells just outside of the blastoporic rim ectoderm, then the roof of the archenteron is ectodermal, but if we prefer to call all the cells that are turned into the archenteron endoderm, then the dorsal wall is endoderm. Chacun à son goût.

A sheet of cells appears between the dorsal wall of the archenteron and the ectoderm. At the posterior end it encircles the blastopore. This is the mesoderm. Whether the cells which make up this layer are rolled in pari passu with the inrolling of the cells around the blastoporic rim (and are continuous with the under-layer of ectoderm), or whether they come from a still deeper layer lying beneath the ectoderm, has not been definitely determined. In early stages, when the dorsal lip begins to form, there is no difference that can be detected between those cells that will form the under-layers of the ectoderm, and those that will become the anterior mesoderm. Since the same condition is present around the lateral lips and at the posterior lip also, we can not state whether or not the early mesoderm is derived from the under-layer of ectoderm at the edge of the blastopore, or from cells present as such from the beginning of invagination. Possibly the mesoderm comes from both sources, and certainly in the later stages immense additions are made to it from the outermost layers of »yolk cells«, by means of which the mesoderm extends around the egg.

It is also probable that a few cells are later added to the mesoderm at the posterior end on each side of the notochord, as claimed by Schultze. The number of these cells appears to be extremely small, so that no important addition to the mesoderm is made from this source, as claimed by Hertwig.

In the preceding review I have attempted to give what appears to me to be, at present, the most plausible interpretation of the facts, and for the sake of brevity have avoided giving a full discussion of all the views that have been expressed in regard to the process of gastrulation in the frog's egg. The bearing of this discussion will be apparent when an examination of the abnormal embryo has been made.

---

[1] Anterior to these cells the dorsal wall may be made by the cells that first pull in from the surface. How far this process goes, and whether, as suggested on page 261, further additions are made at the anterior end, remains to be discovered.

Influence of Gravity on the Egg.

The literature dealing with the influence of gravity on the development of the frog's egg begins with **Pflüger**'s first paper in 1883. Unfortunately **Pflüger** did not detect the real part played by gravity in his experiment, but the methods of experimental study which he initiated opened a new field of embryological research. **Born** showed, in 1885, that in eggs held in oblique positions, as in **Pflüger**'s experiments, gravity acts on the contents of the egg bringing about a readjustment of the parts — the lighter parts moving towards the top of the egg and the heavier to the bottom. Since the outermost layer of the egg does not take part in the rotation, the obliquely placed egg appears to retain its first orientation, as **Pflüger**, erroneously, supposed to be the case.

Roux had shown, in 1884, that there is no necessary, i. e., directive influence of gravity on the formation of the embryo. He showed this by placing eggs on a centrifugal machine. Since the eggs orientate themselves in respect to the centrifugal force it may be objected that under the conditions of the experiment the centrifugal force may have been substituted for the action of gravity. **Roux** foresaw and anticipated this objection, and by regulating the rapidity of the rotation he found the eggs remained in any position in which they happened to lie when placed on the machine. Under these conditions the egg changed its relation to gravity at each moment, and since each egg had a different position in relation to the centrifugal force, both forces were excluded as constant factors. Nevertheless normal embryos developed. In another experiment the eggs were placed in a tube only partially filled with water, and the tube put on a rotating machine. As the tube revolved the water passed from one end of the tube to the other, and the eggs were kept in constant and irregular motion. Still, normal embryos developed. **Roux** concluded that gravity has no necessary, or directive influence on the development.

Into the discussion in regard to this question that has more recently taken place mainly between **Roux** and **Schultze** it will not be necessary to go, since **Kathariner** has recently reviewed the matter very thoroughly and with his conclusions I entirely concur. **Kathariner** himself carried out a few experiments that support **Roux**'s contention. A bunch of eggs was placed in a glass cylinder filled with water. Air was carried through a tube to the bottom of the water, where it emerged in bubbles, which rose to the surface on one side of the cylinder. The rising bubbles caught the eggs and carried them to the surface on one side, and they then sank to the bottom again on the other side of the cylinder, to be caught up again by the bubbles, etc. During its revolution the bunch of eggs turned over and over on itself, so that the eggs constantly changed their relation to gravity. Normal embryos developed. KATHARINER concluded: »The influence of gravity on the normal development of the frog's egg is neither that of a formative force (PFLÜGER), nor of a necessary condition (SCHULTZE).«

We are concerned at present, however, with another side of the question, for although gravity may not have an influence that is necessary to bring about development, yet we can foresee that could its force be increased, or another similar one substituted for it, changes might be brought about in the constitution of the egg that would alter its mode of development. While we can not experimentally increase the force of gravity, we can, by means of a centrifugal force, produce a similar effect in the egg which alters the development. The rate of rotation used by ROUX was too small to produce a change in the development; he had, of course, another purpose in view.

HERTWIG, in 1898, subjected eggs to a much greater centrifugal force, and showed that marked changes take place in the development[1]. He placed receptacles containing eggs on a centrifugal machine. The rate of revolution varied in one series between 140 (at the beginning) and 147 (somewhat later) revolutions per minute. The receptacles containing the eggs were fixed along the radii of the wheel at distances of from 14 to 40 cm from the center of rotation. The eggs of Rana esculenta were put on the machine three hours after fertilization. In the eggs at 40 cm from the axis there was present, a few hours later, a number of furrows in the upper hemisphere, but on the following day these eggs were dead. The eggs at 14 cm distance divided nearly normally, and were in the blastula stage on the following day. Many of these eggs produced normal embryos. Some of them had, however, a very large exposure of yolk in the blastopore. The two series of eggs lying between those just described gave the most interesting results. In most of the eggs the cleavage was normal, at least to the extent of the furrows passing through the white hemisphere, but the later cleavages were confined to the dark hemisphere. This result is due, HERTWIG thinks, to the yolk, which is the heaviest part, being carried into the white hemisphere as a result of the centrifugal action. The lighter parts of the egg remain in or move into the white hemisphere. In consequence of the changes in the interior, the position of the nuclei is affected. The nuclei with their lighter nuclear sap remain in the black hemisphere.

---

[1] RAUBER had carried out in 1884 a similar experiment on trout eggs. Although he rotated them 200 revolutions per minute normal development took place.

Sections of the eggs that have segmented on the machine show the upper third of the egg made up of cells, the rest is occupied by non-cellulated yolk. HERTWIG distinguishes also a subgerminal zone, where the yolk- and cellular parts come together. Here are found numerous nuclei, often large and swollen.

Eggs from the more distal part of the radius may show no division into cells, and only irregular nuclei scattered in a vacuolated fluid. Eggs nearer the axis show an approach to the normal.

During the following day gastrulation takes place in many eggs. The blastopore lies at the black-white border. Many of the embryos that develop from these eggs have protruding yolk in the blastoporic region like those in some of our figures.

A number of experiments were also made with Rana fusca. HERTWIG found when the eggs of this species were put on the machine, soon after they had been fertilized, that they did not develop at all, or died; while eggs placed on the machine just before the first cleavage appeared developed. I may add here that I have obtained similar results, and have found further that eggs which have divided once or twice will withstand a greater rate of revolution than those that have not divided. Moreover eggs that have segmented a number of times, so that the contents is divided by cell-walls, will develop normally at rates of revolution that kill or produce abnormalities in unsegmented eggs, or eggs just beginning to segment.

HERTWIG's results with eggs of Rana fusca were similar to those with Rana esculenta.

In another series of experiments the eggs were treated somewhat differently. Fertilized eggs were placed on glass slides; water added, and the jelly allowed to swell. They were then placed in boxes in which the air was kept saturated with moisture. The boxes were placed on a machine revolving at 200 revolutions per minute. Sections of these eggs show that the cells in the upper part are arranged radially, the point of convergence being the bottom of the segmentation cavity. No explanation is offerred to account for this arrangement.

If we compare these results of HERTWIG with those of my own, which are to be described in the following pages, we find that while in both cases the effect of the centrifugal force seems to have affected the eggs in the same way, yet HERTWIG did not obtain, or at least does not describe the great diversity of forms that I have found. He mentions briefly in a preliminary note ('96), the occurrence of half embryos, etc.

The Effect of Salt-Solutions.

In 1894 it was shown by MORGAN that by adding 0.6 per cent. of sodium chloride to the water in which eggs were developing, ring-embryos (spina bifida) could be artificially produced[1]). In the following year HERTWIG (1895) carried out a more extensive series of similar experiments with stronger and weaker solutions of sodium chloride. Eggs of Rana esculenta were placed, one hour after fertilization, in solutions of 0.5, 0.6, 0.7, 0.8, 0.9, 1.00 per cent. of this salt. Solutions stronger than 0.6 per cent. retarded the segmentation, especially that of the white hemisphere, and the eggs died without going to or beyond the gastrula stage. In the 0.6 per cent. solution the embryos showed well marked abnormalities. The anterior dorsal part of the embryos develops in front of the sickel shaped blastopore. The posterior portion shows a protuberance of yolk. In other words the embryos show the condition, more or less extensive, of spina bifida.

Eggs of Rana fusca are put into solutions containing 0.6 to 1.0 per cent. of sodium chloride, the cleavage process, and the formation of the embryo are delayed. The sides of the blastopore extend slowly around the yolk. When the embryo appears the anterior end of the medullary plate does not roll in to form the medullary tube, but the plate remains open[2]). At the posterior end of the embryo, the lateral lips develop slowly, and often fail to cover the yolk, which is left protruding in this region.

Similar results can be obtaimed, as HERTWIG shows, by raising

---

[1]) Solutions of the salts of Barium, Potassium and Calcium gave similar results.

[2]) As in our Figs. 57 and 58 Plate X. MORGAN ('93) had obtained similar embryos in teleostean fishes by decreasing the amount of salt in the water.

the temperature of the water in which the embryo develops. At a certain point the yolk is more affected than is the more protoplasmic part of the egg. Also by keeping the eggs in water cooled to 0, or —1 degrees similar embryos, showing spina bifida, were obtained, when the eggs are brought back to more temperate water. Over-ripe eggs also, as ROUX had first shown, often fail to segment regularly; the yolk portion not dividing completely. From these eggs ring-embryos, and those showing spina bifida in various degrees are produced. These embryos correspond in all respects to those described by ROUX and by MORGAN.

BATAILLON, in 1901, described the effects of a number of salt-solutions, as well as of sugar, on the eggs of Rana temporaria and esculenta. He obtained embryos with yolk protruding at the posterior end, and in the middle of the dorsal surface (spina bifida) similar to those just described. The effect of salt-solutions at different temperatures, and also their effect on immature eggs (taken from the uterus when only a part of the eggs had reached this organ) are described, and the results discussed. The embryo from eggs not fully matured did not form any clearly recognisable organs, but appear, nevertheless, to have developed cilia over the outer surface. The action of the salts, BATAILLON concludes, is not determined by their chemical, specific qualities, but is to be measured by their molecular weight, and by their isotonic coefficient.

GURWITSCH¹) published in 1895 and 1896 an extensive series of experiments on the action of certain substances in solution on the development of the frog's egg. Some of the halogen salts were found to produce abnormalities in the position and development of the blastopore, and of the brain. Sodium chloride acts on both organs while sodium bromide only on the brain, and lithium chloride principally on the blastopore and on the archenteron, even more definitely than do the other salts.

Lithium chloride, 0.8 and 0.7 per cent., delays the cleavage of the white hemisphere which often remains unsegmented, but a solution of 0.5 per cent., gave the best results. A slow process of cleavage precedes the formation of the blastopore, which appears first as a small invagination. The lateral lips extend around the equator of the egg, and the yolk remains entirely exposed instead of gradually disappearing as in the normal egg. The lateral lips as they grow

---

¹) Anat. Anz. 1895. Archiv f. Entwickelungsmech. 1896.

outwards extend even into the black part of the egg, instead of extending, as in the normal egg, into the region between the black and white hemispheres. Sections show that the surface cells sink in where the blastopore is formed. As the depression deepens the segmentation cavity disappears. At this time the egg, or embryo appears to be radially symmetrical since GURWITSCH could find no differences between the dorsal and lateral parts of the ring. Even the oldest stages observed showed no trace of a ring-formation of axial organs, of mesoderm, or of a union of the lips in the middle line. Nevertheless, a section is given of a later stage in which a medullary plate appears high up on the black hemisphere. Beneath the medullary plate there is a notochord, and below this a part of the archenteron. GURWITSCH gives figures showing a number of transitions between embryos of this sort and those showing spina bifida. The former have many points of resemblances to some of the embryos described in the second part of this paper, and on the basis of what these will show I venture the suggestion that GURWITSCH did not find the real interpretation of this kind of embryo.

GURWITSCH found that strychnine ($^1/_{10}$ and $^1/_{20}$ per cent.) produce spina bifida embryos with a large exposure of yolk at the posterior end. We shall give later a different interpretation of these embryos from that suggested by GURWITSCH. Kaffeïn gave similar results. Nicotin (5 parts to 10.000) gave »typical departures« from the normal cleavage. There is a broad blastoporic rim without a posterior closure. The top of the segmentation cavity is unusually thin. The older embryos have a posterior exposure of yolk, a flat, open medullary plate ending posteriorly in a pair of knobs, and resemble the embryo in our Fig. 52. The chorda was twice the normal size. A solution of grape sugar, 2 per cent. in strength, prevents the development further than the blastopore-stage. A one per cent. solution produced embryos with a posterior extrusion of yolk.

Influence of Lack of Oxygen.

In addition to a few general statements of the necessity of the presence of oxygen for the normal development of the frog's egg, there are one or two experimenters who have attempted to determine this influence more precisely.

SAMASSA ('96 and '98) placed fertilized eggs of Rana fusca in water free from dissolved oxygen, and found that development proceeded for 24 hours. Other eggs were kept in an atmosphere of nitrogen and of hydrogen; they also developed, and when removed after four days and put into fresh water produced abnormal embryos. These showed, for the most part, all variations of spina bifida. In other eggs the medullary plate did not appear. SAMASSA concluded that oxygen is not essential for the development during the first 24 hours. The nitrogen and hydrogen acted, he throught, injuriously on the egg.

BATAILLON ('97) measured the amount of oxygen used during development, and studied the influence of carbon dioxide on the egg. He obtained results similar to those of GODLEWSKI whose results are given below.

SCHULTZE ('99) found that in the absence of oxygen for 24 hours the early development was retarded, and in some cases the eggs were killed. It has been pointed out that since the carbon dioxide was not removed in this experiment, the results may have been due in part to this. GODLEWSKI has shown, in fact, that carbon dioxide is injurious to the egg.

GODLEWSKI ('97, 1900, 1901) carried out a more elaborate series of experiments, and found that the rate of development is closely connected with the presence of oxygen, although the cleavage may proceed more or less normally, without oxygen being supplied from the outside. The effect of increasing the carbon dioxide is noticeable even from the beginning of development. It exerts a specific, toxic action on the developing egg. GODLEWSKI does not describe embryos having spina bifida as a result of the absence of oxygen, but his experiments were not carried out with this end in view.

BATAILLON (1901) called attention to the similarities of his results and those of GODLEWSKI.

Effect of Temperature.

HERTWIG ('94, '96, '98) has carried out numerous experiments on the effect of temperature on the development of Rana fusca and esculenta. For the former he found the optimum temperature for development to be in one series 22 degrees, in another[1]) 24 degrees. Above these temperatures, for instance at 27 to 29.6 degrees, irregular segmentation of the dark hemisphere takes place, and the eggs die without producing embryos. Between 23—24 degrees, and between 25—26 degrees, most eggs die; some produce abnormalities, and only a few become normal embryos. The abnormalities were of two sorts. First those with spina bifida; second, those with deformed heads, often combined with posterior spina bifida.

---

[1]) The time elapsing between fertilization and the removal of the egg to the low temperature probably accounts for the difference in the two cases.

The eggs of Rana fusca were fertilized at a temperature of 9 degrees, kept for two hours at this point, and slowly ($1^1/_2$ hours) cooled to 1,5 degrees. The early development extended over weeks and even months. It took thirty days before the blastopore closed.

No abnormalities were observed, although HERTWIG thinks that the embryos were more or less injured and would not have become normal tadpoles.

In another experiment in which the eggs were fertilized and placed soon afterwards in water at 7 degrees, then at 6 degrees and so on to 1,5 degrees (the transfer being quite rapid) the eggs began to develop, but abnormally. They became more and more abnormal, and in most cases died. Even those kept at 2,5 degrees, although they reached the morula stage, died later, and this is true also for eggs kept at 4 degrees. Some of those at 6 degrees had after 8 days developed to a stage with a small round blastopore.

The difference in the results of these two series of eggs of Rana fusca is to be explained by the more gradual change after fertilization in the former series which suffered, therefore, less from the cold. HERTWIG explains the difference between his results and those of SCHULTZE ('99) as due to the different time at which they subjected the eggs to the cold. SCHULTZE used later stages, and found that although the eggs developed slowly, yet the development was normal.

HERTWIG found that the optimum temperature for eggs of Rana esculenta (which are laid a month later than those of Rana fusca) is higher, viz., 32—33 degrees.

HERTWIG also carried out experiments to determine the influence of temperature (on each side of the optimum) in regard to the rapidity of development, but these results have no bearing on our present subject.

In regard to the effect of temperature on the eggs of Rana palustris I have found that if eggs, in the first or second cleavage-stage, are put in an ice-chest the cleavage is stopped, and the eggs may die in the course of a few days. If the eggs in the blastula-stage are kept at the same temperature they withstand the cold much better, but if kept for several weeks they give rise to abnormalities. The gastrula-stages withstand the cold better, and the development may proceed with extreme slowness. If embryos in which the blastopore has closed and the medullary folds have rolled in are kept at the same temperature they will remain in good condition for more than a month. The advance in their development is scarcely perceptible, but still it goes on very slowly. If the embryos are taken out and placed in warmer water, they give rise to normal tadpoles.

The eggs of Rana temporaria (sylvatica) withstand the cold much better, and even the segmenting eggs can withstand the temperature of freezing water for several days or longer. It is interesting to note here also that the eggs of Rana temporaria are laid very early, almost as soon as the ice has melted, and are often caught by a later freezing. If they are not actually caught in the ice they recover. The eggs of Rana palustris are laid several weeks later and are, as just stated, more sensitive to cold especially in the early stages.

Part II. Experimental.

Abnormalities Produced by Centrifugalizing the Eggs.

The eggs of Rana palustris were collected from the pond in which they had been laid and fertilized under normal conditions. It is worth while to emphasize this point, since it means that at the beginning of the experiments the eggs were as nearly normal as is possible to have them. This will not always be the case, as every experimenter must have found, when eggs are artificially removed from the uterus and artificially fertilized.

In some cases the eggs had not yet segmented, others were in the two and four cell stages, etc. They were placed at once on the centrifugal machine, which was the large driving wheel of an engine. The eggs were placed in glass bottles $4^1/_4 \times 1^3/_4$ inches filled with water. About fifty to one hundred eggs were in each bottle. These bottles were put into wooden cylindrical tubes, closed at one end and with a screw top at the other[1]. The boxes were fixed with wire along a radius of the wheel. The box nearest the center of the axis on each radius was, at its inner end, about $4^1/_2$ inches from the center of rotation. At its outer end $7^1/_2$ inches (inside). The next box, standing on the end of the first, was at its inner end about $10^1/_2$ inches from the axis, at its outer end $13^1/_2$; the third box $16^1/_2$ at its inner end, and $19^1/_2$ inches at its outer.

---

[1] These tubes were, in fact, ordinary mailing tubes.

The wheel was started at first slowly for a few seconds and then allowed to go faster and faster until in about a minute the required speed was attained. In the first series the rotation was found to be too slow for eggs that had begun to segment. In later experiments the eggs were often revolved 180 revolutions a minute, at which speed some of the eggs were so injured that they failed to develop; others, however, gave rise to interesting abnormalities.

Two conditions in these experiments must be carefully noted. It was found that eggs that had not segmented were more easily killed than those in the two and four cell stages. If, for instance, eggs were taken from a bunch in which under normal conditions the eggs did not segment for several hours, they would be killed if rotated at the same speed as that used for eggs in the two and four cell stages.

In most of the experiments the eggs were going into the two cell stage, sometimes into four cells. It should be noted that in both cases the continuity between the upper and lower parts of the egg is still unbroken by cell-walls.

The other important condition is the distance of the eggs from the center of rotation. If the eggs in the first box are carried by the centrifugal force to the outer end of the bottle they are subjected to a centrifugal force about 7 times that of gravity[1]). When the box is at the lowest part of its revolution gravity and centrifugal force act together and in the same direction. When at the highest point the two act in opposite directions, and at intermediate points at varying angles. The rate of revolution was so great, that it is certain, to judge from what others have observed, the eggs orientated themselves so that the white hemisphere was turned outwards, and remained in this position throughout the entire rotation.

It was found in those sets, in which after removal from the wheel the different lots were kept apart, that those in the outer boxes were, of course, the most affected. Since, however, my object was to study the effect of the centrifugal force on eggs brought as nearly as possible to the fatal point at which development is stopped I arranged the experiment, in most cases, so that the eggs in the outer sets would be seriously injured, and those nearer the axis not so much affected. These gave most of the abnormal forms figured in this paper.

---

[1]) Eggs in the outer part of the second bottle were subjected to a centrifugal force about 13 times that of gravity and in the third bottle about 19 times.

The eggs were left on the wheel from 7 to 10 hours. When taken off they were put into flat dishes with plenty of water, which was, as a rule, changed every day. During the time they were on the wheel, the eggs continued to divide, although the number of cleavage furrows which cut through in the lower hemisphere was less than in the normal. In some cases the unsegmented egg did not divide; and sometimes eggs that were in the two and four cell stages were so injured that they did not divide further[1]. Check series of eggs from the same bunches were in a few cases kept in similar bottles, closed in the same way as those on the wheel. In these, the eggs developed normally showing that the enclosure for 7 to 10 hours in this limited space had nothing to do with the production of abnormalities. The room in which the engine worked was warmer than the average temperature outside, but not so high as to injure the eggs[2]. In consequence of the warmth, the eggs on the wheel that were not much injured developed faster than the check eggs kept in another room.

The following records will serve to give an idea of the effect of the centrifugal force on the eggs.

I. Series. Unsegmented eggs were put on the machine at 9 15 A. M. The check eggs segmented an hour later. The rate of revolution was at first 150 per minute. A little later 170 per minute. The eggs were left on the machine until 9 30 P. M. When taken off a pigmented ring had formed just above the equator of the egg, the upper hemisphere being much lighter in color than normal. Most of the eggs died, but a few in each set produced the most extreme forms of abnormalities.

II. Series. Unsegmented eggs were put on the machine at 10 A. M. The rate of revolution was at first only 100 per minute. Later 120 per minute. The eggs were left on the machine until 5 30 P. M. Next day all eggs gastrulated normally. The rate of revolution was obviously too slow.

III. Series. There were two lots in this experiment. In one lot the eggs had between 250 and 500 cells; in the other lot the dorsal lip of the blastopore had just appeared. They were on the wheel for four hours, making 140 revolutions per minute. Gastrulation went on normally, and no abnormalities were produced.

---

[1] Or if they did the furrows were obliterated.

[2] As shown by their normal development when not rotated too fast.

IV. Series. Four different lots of eggs were used. Two of these began to segment when put on the wheel; the other two after half-an-hour. They were put on the machine at 9 45 A. M. and revolved at the rate 130 revolutions per minute.

At 11 05 A. M. the machine was stopped for five minutes, and two new lots were put on, some in the two cell stage, others in the four cell stage. The machine began again with 140 revolutions per minute. The eggs were taken off at 6 P. M.

From this series many spina bifida embryos were obtained. Some of the embryos were half-embryos. Some lots (not specified) developed normally. No observed differences were recorded between the unsegmented and those in the two cell stage; but it should be noted that the unsegmented were just about to go into the two cell stage.

It was not from the preceeding series that the embryos, here described, were obtained, although some of the same kinds were noted in both cases. Most of the material was selected from the following series.

V. Series. Eggs at about the 128-cell stage were kept on the machine seven and a half hours, revolving 180 revolutions per minute. Many spina bifida embryos were produced as well as other abnormalities.

VI. Series. Eggs in the two-cell stage were kept on the machine about seven and a-half hours, revolving 180 revolutions per minute. This series as a whole gave excellent results.

VII. Series. Eggs in the two-cell stage were kept on the machine about seven and a-half hours. These gave equally good results. The wheel made 150 turns per minute.

VIII. Series. Eggs in the two-cell stage were kept on the machine for $6^1/_2$ hours. These also produced many abnormalities. The wheel made 180 revolutions per minute.

The following four series are from another experiment.

IX. Series. Eggs going into the two-cell stage were kept on the machine for eight hours, making 154 revolutions per minute. Excellent results were obtained.

X. Series. Some of the last lot were kept on ice over night. They were in the four cell stage when put on the machine, which made 160 revolutions per minute. Three lots gave many abnormalities; in one lot all the eggs died. In this same experiment, other eggs were put on the machine that had been segmenting for 24 hours. These gave normal embryos.

XI. Series. Unsegmented eggs were put on the machine and rotated at the rate of 160 revolutions per minute. Nearly all died without producing embryos. Curiously enough one egg made a nearly normal embryo. The check eggs did not divide until seven hours after the others were put on the machine.

XII. Series. Eggs from the last series which had segmented over night were put on the machine for eight hours. They were revolved 180 revolutions per minute, and produced many abnormalities like those of the other two-cell sets. One lot went normally. In this same series another lot was unsegmented. It remained eight hours on the machine. Most of the eggs died.

The embryos from which those represented in Fig. 1 to 47 were selected, belonged to set V (April 18). The eggs were in the 128-cell stage when put on the wheel. In looking over the twelve series I picked out this one as showing most clearly the greatest number and variety of forms. A comparison of the embryos of this set with those of others, developing from eggs that had been put on the machine in the two- and four-cell stages, shows that the principal forms here described can be duplicated in other sets. As far as the end-result is concerned it makes little difference which set is selected, but there are certain obvious advantages in having the different kinds of embryos all from the same set.

It was my intention at first to arrange the different kinds of embryos, figured in the plates, in groups classified according to their resemblances. It would not, in fact, be difficult to arrange the series so that in passing from one end to the other one would find everywhere connecting forms, but after some consideration I have come to the conclusion that such an arrangement might lead to a misconception of what the results have shown. The figures stand, therefore, in the order in which they were picked out in lots of fours or fives to be drawn and sectioned. The reader who is not especially interested in the details will find the description of a few of these embryos sufficient and will pass on to the general conclusions at the end.

Description of Embryos.

No. 1. The surface view, Fig. 1 Plate VIII, shows the dorsal part of the embryo lying along the border between the black and white portions. The embryo bends to one side just behind the brain region. A cross-section anterior to the middle of the embryo is shown in Fig. V Plate XI. The ectoderm extends much further down on one side than on the other (right and left are always reversed in the surface drawings as compared with the sections; except when otherwise stated). The ectoderm is a little thickened along the free edge, but otherwise forms no definite structure along the border. At a posterior level the lower edge of ectoderm turns in near the anal depression. The nervous system is symmetrical, but small. Below it lies the notochord. The archenteron is a small space lying below and to one side. Mesoderm is present on each side of the median line, and equally developed on each side. In the yolk there is a large hole. Only that part of the yolk lying near the embryo contains nuclei.

No. 2. The next embryo, Fig. 2, shows what appears to be a half-embryo except at the anterior end which is more than half. A cross-section through the middle of this embryo is represented in Fig. IV Plate XI. The nerve tube is closed and rounded (posteriorly it is open and is incomplete on one side). It has been formed exactly along the border of the yolk. Beneath there is a small notochord, and on both sides of the latter mesoderm is present; but that on the yolk side is much less in amount than that on the other. The archenteron is a very small cavity just below the notochord (anteriorly it is larger). The ectoderm ends abruptly below. Beneath the ectoderm and along the side, a layer of mesoderm is present. There is a sharp contrast between the nucleated and non-nucleated yolk.

No. 3. In this embryo, Fig. 3, only the dorsal region is developed. The ectoderm extends only a short distance on each side of the mid-dorsal line. At the anterior end the embryo is more nearly pinched off from the injured yolk, as shown in the cross-section, Fig. I Plate XI. Here a sharp line is present between the nucleated and non-nucleated yolk. In the middle region of the embryo, Fig. II, the embryonic part is less sharply separated from the non-nucleated yolk. Towards the posterior end the mesoderm is less developed on one side Fig. III. A small archenteron is present. The sections show clearly that only the dorsal side of the embryo has developed; and the free edge of the ectoderm on each side shows nothing to suggest that it is a blastoporic rim.

No. 4. The surface view of this embryo, Fig. 4, gives the appearence of a half-embryo, but sections show that the embryo is more nearly a whole embryo of half size. The section that is drawn in Fig. VI Plate XI, is through the middle region, and shows a closed nerve tube, a round notochord, and an excentric archenteron near the edge of the nucleated yolk. It is important to note that the mesoderm on each side of the nerve tube and notochord is equally developed on both sides. Below this level it is only present on one side, the outer, where it is differentiating from the yolk. A large part of the egg takes no share in the formation of the embryo. It is also important to note that there is no indication of a blastopore-lip at the free edge of the ectoderm.

No. 5. The next embryo, Fig. 5, appears in surface view to represent the right half of an embryo; and cross-sections, Fig. VII Plate XI, show that this is the case. Only a small part of the egg goes to form the half-embryo. The nerve tube that has just closed is imperfect on one side, and represents nearly exactly half-a-tube. The notochord lies near the free edge. The archenteron is a very small hole just below the notochord. The mesoderm is present only on one side. The lower, free edge of ectoderm shows, perhaps, indications of its being a blastoporic rim, inasmuch as there is a thickening of mesoderm, and an inturning of cells in this region (at other levels than that of the figure). Only that part of the yolk that lies near the embryo is organized; the rest is non-nucleated, vacuolated, and appears more or less dead.

No. 6. The surface view, Fig. 6, shows a broad, but short dorsal part of an embryo, which occupies only a small part of the egg. A cross-section through the middle of this embryo, Fig. VIII, shows a closed nerve tube, a small notochord beneath, with mesoderm on each side, but less developed on the yolk-side. The archenteron is a triangular space below the notochord. The organized, nucleated yolk is fairly sharply separated from the rest of the yolk.

No. 7. The embryo represented in Fig. 7 and 7*A* forms an interesting contrast to those shown in Figs. 3 and 6, since it represents largely only the ventral surface of the embryo. In Fig. 7 the anterior end, as seen from the dorsal point of view, is shown. What appears to be a blastoporic rim extends around each side of the egg. When viewed from below, Fig. 7*A*, a pair of suckers is seen at the anterior end, and the figure is so turned that one side of the blastoporic rim can be seen. Sections through this embryo, Figs. IX and X, show that a nerve tube and notochord are formed only along one side of the blastoporic rim. Along the opposite side there is scarcely any evidence suggesting the presence of a half-ring, except the thicking of mesoderm, and an indentation of the edge (with a split in the yolk along this side). A curious collection of cells just below the indentation (in Fig. X) may possibly represent the notochord. The side along which the embryo has developed has produced more than a half-embryo, as seen in Fig. X, since mesoderm is present on both sides of the notochord. The embryo suggests a ring-embryo with only one-half of the ring developed, and even overdeveloped.

No. 8. The embryo shown in Fig. 8 is less advanced than the preceding embryos, and has a bilateral anterior end, while the posterior end is only half an embryo. Attention should be drawn to the embryonic edge on the left side which corresponds to the missing half of the embryo. It has remained undeveloped. Sections add but little to what is seen in surface views.

No. 9. Here also, Fig. 9, the posterior end is incompletely formed on one side, owing, it appears, to the lack of development of the embryonic rim of that side. The sections add nothing of interest.

No. 10. In this case, Fig. 10, although the blastoporic rim has failed to grow over any considerable part of the yolk, yet the medullary plate is developed on both sides; but at the posterior end it is more complete on that side (the left) which appears to have advanced over the yolk. This embryo produces the impression of having formed its whole posterior end out of only one side of the blastoporic rim. It would have resembled probably in later stages the embryo shown in Fig. I.

No. 11. This embryo, Fig. 11, appears in surface view to be somewhat more than a half-embryo. It is in a younger stage than are the half-embryos shown in Figs. 2 and 5. A cross-section through the middle of this embryo, Fig. XI, shows the open medullary plate ending at one side in a sharp edge. Just below this edge lies the notochord, and to the right of it there is a narrow archenteric split in the yolk. There can be no doubt that the lateral blastoporic invagination took place along this line. The mesoderm is developed only on one side. The lower free edge of ectoderm ends abruptly, and there is no evidence of a blastoporic rim in this region. A large fluid space is present in the undeveloped yolk.

No. 12. Only a small part of the egg has developed in this case, Fig. 12. A short, rather broad medullary plate is closing in. A cross-section, Fig. XII, shows the greatly thickened ectoderm, the medullary plate nearly symmetrical at this level, a notochord beneath and a narrow archenteron. The nucleated yolk is, for the most part, sharply separated off from the rest.

No. 13. The surface view, Fig. 13, shows a part of the anterior end of an embryo. Sections confirm this interpretation, Fig. XIII, showing a thick medullary plate in the anterior part. For a short distance a notochord-like structure is present. Along the edge bordered by the nerve-plate a split is present. It is the archenteron.

No. 14. A small part of the egg has produced an embryo, Fig. 14. A cross-section through the anterior end, Fig. XV, shows that the organized part has nearly constricted off from the rest of the egg. The nerve-plate has not yet rolled in. At the posterior end, Fig. XIV, the embryonic portion is not so sharply marked off from the yolk. The embryo is somewhat less developed on one side than on the other.

No. 15. The surface view, Fig. 15, shows a cake-like dark portion with a distinct rim, which separates the dark part from the yolk on which it rests. No evidence of an embryo was seen, but sections show that a medullary plate is present at one side, Fig. XVI. Beneath it lies a very short notochord. Only a very small region of the yolk is nucleated, as shown in the figure. It lies next to the embryo.

No. 16. In surface views this embryo shows a broad anterior medullary plate, Fig. 16, with a large exposure of yolk behind it. On the other side of the egg, Fig. 16*A*, there could also be seen, in surface view, a thickened region resembling a medullary plate. The surface views suggested two embryos, turned in opposite directions, each with its posterior end abutting against the yolk. Sections show, however, that this is not the case, for while the anterior thickening (Fig. 16) is undoubtedly a medullary plate (with a notochord beneath), the posterior thickening does not resemble the anterior end of a medullary plate. Where the latter thickening abuts against the yolk there is an anal-like inturning of ectoderm. The thickening itself can not be satisfactorily accounted for, unless it represents the flattened tail-knobs.

No. 17. In this embryo, Fig. 17, the medullary plate extends along the side of the free edge. In others words, a whole medullary plate has formed along one side of the blastoporic rim. The sections were not good, and gave nothing further.

No. 18. In Fig. 18 the embryo shows something more than a half-structure on the dorsal side. On the less developed side the rim has not extended as far backwards as has that of the other side.

No. 19. A whole medullary plate is present along one side of the blastoporic rim, Fig. 19. A cross-section through the middle of this embryo, Fig. XVII, does not show the medullary fold so strongly marked as does the surface view. A notochord lies beneath the medullary plate, and beneath this there is a well marked archenteron which opens along the side between the edge of the medullary fold and the yolk.

No. 20. The anterior end of the embryo is broad and well formed, Fig. 20, and lies far forward on the black part of the egg. The posterior end is narrower and projects backward over the surface of the yolk. A longitudinal section, Fig. XVIII, of this embryo was made in order to see if there were any signs of a blastoporic rim along the anterior edge, or along the sides. There is nothing present at the anterior edge to suggest either an inturning of cells, or an increase of mesoderm. In fact, other embryos of this sort show that the anterior edge is on the same side of the segmentation cavity as that on which the embryo is formed.

No. 21. Here we find something more than a half-embryo, Fig. 21, but yet not a whole one. A cross-section, Fig. XIX, through the middle of this embryo shows a broad medullary plate, about two-thirds full breadth. The opening of the wide archenteron is along one side of the plate.

No. 22. Another embryo, Fig. 22, similar to that of Fig. 20, was also cut longitudinally. The median section, Fig. XX, shows the dorsal embryonic region lying above a large archenteron which opens out at the posterior end. A large cavity in front of the anterior end of the embryo is probably the segmentation cavity. Only that part of its upper wall that is on the embryo-side of the egg is developed. The opposite side is dead, or undeveloped, as is also the yolk on that side of the egg.

No. 23. The surface view, Fig. 23, shows a whole embryo of half-size (or less) open on the side next to the yolk. A cross-section, posterior to the middle of this embryo, Fig. XXI, shows that it is not quite as complete as appears in surface view, since the nerve-tube is smaller on one side, and the mesoderm on this side is very little developed. The archenteron is a narrow excentric split. The anterior end of the embryo is complete, and both sides equally developed. The region posterior to this section is even more incomplete on one side than it is at the level of the section.

No. 24. In this embryo, Fig. 24, the anterior end of the embryo is well formed and symmetrical, but at the edge of the black border it takes a turn to one side, and continues along the blastoporic rim. The other side shows no signs of the formation of an embryo. The sections were cut in an oblique plane and add nothing to what can be seen in surface view. The anus is found on the lower side of the egg at about the level where the embryo above turns to one side. The anus lies, therefore, far removed from the posterior end of the embryo.

No. 25. The entire anterior end of an embryo is shown in Fig. 25 with a widely open posterior part. The anus, as shown by the sections, does not lie at or even near the posterior end of the nerve tube, but nearly on the opposite side of the egg. It lies on the black border near the lower left hand side of the figure, where a considerable thickening of mesoderm is present. The rest of the border shows some signs of being a blastoporic rim — at least for the greater part of its extent, since the edge is slightly tucked in, and the mesoderm that is developed along the edge is continuous with that of the embryo.

No. 26. This embryo, Fig. 26, is in some respects similar to the last. It is shown in side view. It was sectioned in a horizontal plane in order to examine the rim along the sides. On the right side, — that shown in the figure — the mesoderm is thicker along the rim, and about half way down the anal invagination is present. Below this the rim is like that of the other side. Along that side the edge of the ectoderm is rounded and the mesoderm comes down to the edge. The edge seems to be a blastoporic rim, but it should be noted that there is no actual archenteric inpulling or inturning of cells.

No. 27. In this embryo, Fig. 27, the medullary plate has just rolled in; the dark part, lying on the yolk, represents the dorsal region only of the embryo, Fig. XXII. On each side of the medullary plate the ectoderm extends over the yolk; further on the left side than on the right. There is no indication that the edge of ectoderm is a blastoporic rim. Beneath the closing medullary plate there is a large notochord and the archenteron is below this. The embryo is unique in several respects. The ectoderm of the medullary folds (especially the one on the yolk-side) is lighter in color than the rest of the black area, and this is due to the presence of yolk cells just beneath it, as shown at one place in the section. The brain region is little developed. There is some evidence pointing to the beginning of a regeneration of the right side; thus obliterating the line along which the archenteron was turned in.

No. 28. The next embryo, Fig. 28, has the anterior end only developed. Horizontal sections show that the lateral edges have none of the peculiarities of a blastoporic rim, except on the side opposite the posterior end of the embryo (the part that is below in the figure) where an inturning of ectoderm indicates the place of formation of the anus.

No. 29. The anterior end of this embryo, Fig. 29, is symmetrically formed and separated from the yolk. A cross-section through the middle of the embryo is shown in Fig. XXIII. The nerve-cord and notochord are small, whole structures, but the dorsal mesoderm is less developed on the open side of the embryo, and this condition persists to the posterior end, where the mesoderm of the tail knob is very much developed, but more so on one side than on the other. The embryo is probable a late stage of one like that shown in Fig. 21.

No. 30. In this embryo, Fig. 30, only a small part of the egg is organized. It has formed a short embryo which, for the most part, represents an anterior end. Cross-sections, Fig. XXV, show the nerve tube closed or closing (posteriorly) and nearly symmetrical. Mesoderm is present on both sides, especially at the posterior end.

No. 31. This interesting embryo, Fig. 31, is not difficult to interpret. Only a very short anterior part of the brain lies in front of the meeting of the two sides of the blastoporic rim. A notch, or dark line in the figure indicates the posterior end of this region. Along the left side of the rim a half-medullary tube is present, as seen in cross-section, Fig. XXVI. A small round notochord lies beneath. The mesoderm on the yolk-side is very little developed, being represented by a small collection of cells. The anterior end of the archenteron is shown in this section, and a single »sucker« on the ventral surface. The ectoderm ends below in a free edge which seems to be extending over the yolk. If this edge is followed into the more posterior sections it will be found to enclose a rounded mass of mesoderm cells, but shows no evidence of forming a part of an embryo on this side. Still further posteriorly the anal invagination is found at the edge; and further back this edge becomes continuous with the other. It is clear in this case that only one half of the blastoporic rim has produced a half-embryo.

No. 32. A broad, open medullary plate appears on the black part of the egg, Fig. 32. Posteriorly it projects as a plate over the surface of the yolk. A cross-section, Fig. XXVII, through the posterior part shows the projecting plate is made up of ectoderm above, a notochord beneath, and mesoderm on each side. The ectoderm is not continuous at the sides with the mesoderm. A sheet of entoderm lies between the plate and the yolk. The sections indicate that this posterior plate is a backgrowth of the posterior end of the embryo, and is not formed in situ from the material in this region, and has no connection with the yolk beneath. In the lower part of the section the ectoderm of the opposite side is present.

No. 33. The surface views, Figs. 33 and 33 *A* (anterior end), show nearly a whole medullary plate laid down along one side of a blastoporic rim. The embryo is in an early stage, and there can be little question of a lateral secondary regeneration. The cross-section, Fig. XXVIII, confirms this view.

No. 34. This embryo, Fig. 34, is, in some respects, like the last, but in a later stage of development. The embryo is turned to one side, and extends along the edge of the blastopore. No sections were made.

No. 35. A quadrilateral cake of black lying on a lighter yolk, Fig. 35, is all that indicates an embryo. Cross-sections, Fig. XXIX, show along one edge (probably the upper one of the surface view) a half-embryo has been formed. The half-medullary fold is just rolling in. Near the edge is the rounded notochord, below which is the archenteric split. The mesoderm is absent on one side. A relatively small part of the yolk (next the embryo) is organized; the rest is filled with vacuoles, swollen nuclei and spaces filled with red-staining granules. At the anterior end the nervous system is nearly complete on both sides, and is separated from the yolk.

No. 36. This embryo, Fig. 36, is a small half-embryo. The head is, however, nearly complete and pinched off. Behind this the nerve-cord is nearly complete, but there is only a very little dorsal mesoderm on the yolk-side. Farther posteriorly the cord is less rolled in, and is less complete on the yolk-side. There are only a few mesoblast cells on this side. Along the lower edge of ectoderm the mesoderm is thicker, but there is scarcely any other sign that the edge is a blastoporic rim.

No. 37. This embryo, Fig 37, is a half-embryo. Sections show that the anterior end is nearly complete, and partly pinched off from the yolk. In the middle and posterior part the medullary tube is rolling in, and is something more than a half-structure. The notochord is near the edge, and near it there are traces of a little mesoderm on the yolk side. The mesoderm on the other side is well developed.

No. 38. The anterior end of a medullary plate has formed in this embryo Fig. 38. Its posterior end is at the same level with what appears to be a blastoporic rim extending around the middle of the egg. The sections were in a somewhat oblique plane. They show that the ectoderm does not turn in at the rim opposite the embryo or at the sides. The mesoderm is much thicker along one side than along the other.

No. 39. In this same series some of the eggs were killed at an earlier stage, — before the embryo had appeared. In Fig. 39 a distinct line is present around the dark border. The region at which the embryo would appear is the upper part of the figure. A longitudinal section of the egg (vertical in respect to this figure) shows that the dorsal lip has advanced some little distance over the yolk, and there is a narrow archenteric cavity reaching up to the level of the segmentation cavity which is still present. The opposite edge of the black portion (on the opposite side of the segmentation cavity) does not extend beyond the level of the segmentation cavity. It is slightly turned in, but abnormal. The conditions are similar to those shown in Fig. XXX.

No. 40. Another gastrula-stage is shown in Fig. 40. Here the edge between the dark and light is higher up than the equator of the egg. Sections of this egg are like those of the last one.

No. 41. The upper part of the rim, Fig. 41, is the dorsal lip which sections show has covered already some distance of the surface of the egg; or at least there is present a long archenteric split, as shown in Fig. XXX; from which I infer that an overgrowth may have taken place. The yolk cells at the anterior end of the archenteron project into the segmentation cavity, as though they had pushed into it. On the opposite side of the segmentation cavity the ectodermal roof stops abruptly and a few scattered nuclei lie near by in the yolk.

No. 42. The black-white rim extends around and above the equator of the egg Fig. 42. Sections, Fig. XXXI, show that the archenteron has just begun to develop, and the pulling in of the yolk cells to form the invagination is, in some places, very apparent. The cells over the roof of the segmentation cavity reach only to the far side, where numerous irregular nuclei lie in the yolk. The free black edge of this side has, therefore, nothing to do with a blastoporic rim.

This fact throws a good deal of light on several of the preceding cases, since it shows that the anterior black-white rim may sometimes have nothing at all to do with a blastoporic formation, although it may be continuous at the sides with the latter, as shown in Figs. 27—30. This point will be more fully discussed later.

No. 43. A diminutive embryo appears on the black part of the egg, Fig. 43, from which a tail-like extension of the medullary folds grows backwards over the yolk. A cross-section through the anterior end, Fig. XXXII, shows a nearly symmetrical medullary fold. The mesoderm is developed only on one side. The ectoderm appears to have grown secondarily somewhat over the yolk on the side. Posterior sections show that the archenteron must have turned in along the side — at least of the more posterior part. One of the more posterior sections, Fig. XXXIII, shows that the medullary plate is incomplete on one side, and the mesoderm is also less on this side than on the other. The anal invagination, as shown in this section, is far removed from the embryonic region. It lies nearly on the opposite side of the egg along the lower rim of ectoderm. Cross-sections of the tail-like extension show that the medullary plate is open and incomplete on one side. Below it are a notochord and mesoderm.

No. 44. This embryo, Figs. 44 and 44*A*, was a complete puzzle until sectioned. As seen from one side, Fig. 43, a large yolk-mass is nearly, but not completely surrounded by a black band. At one point the band is thinner, and a »sucker« is present in this region. When turned over, Fig. 32*A*, the egg presents nearly the same appearance. Cross-sections of the egg show that while the two bands are separated at one end by the injured yolk, they more nearly come together in the region of the »sucker«. One of the cross-sections is shown in Fig. XXXIV. On the right side the anterior end of the embryo is seen. This region is in front of the anterior end of the nervous system, which appears on this side a few sections further back. A large pharyngeal cavity is present surrounded by organized, nucleated yolk, which is sharply separated off from the undeveloped yolk. On the opposite side of this section the other band is cut across, and in it the anal invagination is seen (it opens to the exterior in other sections). A more posterior section is shown in Fig. XXXV. The nerve tube (to the right) is closed. Beneath it lies the anterior end of the notochord, and below this is the archenteron in a nucleated yolk-mass. On the opposite side of the section a mass of cellulated yolk, covered by a sheet of ectoderm, is present. The yolk between the two regions is much injured. Fifteen sections behind this the nerve-tube ends as well as its band. The opposite band ends even sooner.

It is evident that this embryo is in two pieces that can scarcely be said to be united at all except by injured yolk. One piece contains the archenteron and a short nervous system and notochord. The other the anal invagination. The interpretation is as follows. One of the white surfaces, shown in one of the surface views, Fig. 44, is the yolk of the lower hemisphere. The other, Fig. 44*A*, is the dead roof of the segmentation cavity. The two bands are, therefore, equatorial rings that have never united posteriorly, while anteriorly only a slight connection exists in the region of the »suckers«.

No. 45. What appears to be only a portion of an individual has developed in this case, Fig. 45. A curved sucker is the most conspicuous object in surface view. A section nearly at right angles to the length of the sucker shows that only the brain end of the nervous system is present, Fig. XXXVI, which extends through only a few sections. A large archenteron (pharyngeal cavity) is present in other sections.

No. 46. A very small triangular black area appears to represent an embryo of some sort, Fig. 46. Sections show that a nerve-cord has developed along one side. A section through the more anterior region is shown in Fig. XXXVII. Only a very small part of the egg is organized, the rest being much vacuolated. The archenteron opens along one side of the black area. A rounded nerve tube (brain) appears in this section, and below it a collection of cells represent the mesoderm. A few sections further back the nerve tube is open, Fig. XXXVII *A*, and is obviously an incomplete structure. Still further back a notochord is present below and at one side of the nerve-tube, Fig. XXXVII*B*. At the posterior end, *C*, only the nerve-plate is found. In this case it is evident that a piece of an embryo has developed. The piece represents little more than half of the dorsal region.

No. 47. An embryo lies along the edge of the black area, Fig. 47. Both medullary folds are present, but the one on the side next the yolk is smaller and lighter in color. A cross-section through the middle of this embryo, Fig. XLIV, shows the medullary plate bent in and ending abruptly on the yolk side. Beneath it lies the notochord with a small amount of mesoderm on the yolk side. The archenteron opens along one side of the embryo to the exterior. The segmentation cavity is present in the lower part of the section. The ectoderm forming its roof ends abruptly on the side opposite that on which the embryo lies.

No. 48. The following four cases (as well as the last one, No. 47) are from another series, the first of these, Fig. 62, shows a very small embryo lying obliquely on the black part of the egg. Sections show, Fig. XLV, that the embryonic part lies to one side of the old segmentation cavity whose upper wall is composed of dead, or degenerating cells. The opposite side is undeveloped and contains swollen nuclei and pigment. The embryo is not very well developed. The archenteric ingrowth seems to have been along one side. Nearly the whole egg, except the embryonic area is degenerating, even much of the black part itself is disorganised.

No. 49. This embryo Fig. 63 is more than half, and the medullary fold on the yolk side is lighter in color and may be regenerating from the other half. A cross-section, Fig. XLVI, shows something more than half an embryo with the inner medullary fold less developed than the other. The archenteron has turned in along the black-white edge. The pulling in of cells to form the anal invagination is, at the free edge of the ectoderm, opposite the embryonic region at the level, in the figure, where the ectoderm ends below, but somewhat further back.

No. 50. This embryo is also relatively small and lies obliquely on the black area, Fig. 64. Cross-sections show that the open medullary plate is less developed on the inner side. The notochord is somewhat excentric and there is less mesoderm on the inner than on the outer side. A cross-section through the tail end, Fig. XLIII, shows the thickened ectoderm above (it is the medullary plate), the notochord below, with mesoderm on each side. Nearly opposite this region there is a small patch of ectoderm which ends a few sections further back, and just beyond it the yolk cells have drawn in to form the anal invagination. The position of this invagination is shown in surface view in Fig. 64*A*. It lies at the upper pointed free end of the ectoderm. The invagination lies nearly 180 degrees from the posterior end of the embryo, but continuous, organized material connects the two parts.

No. 51. This embryo, Fig. 65, is extremely abnormal and consists of the anterior end of the medullary plate from which there has grown backwards a tail-like extension over the yolk. Sections show a notochord beneath the medullary plate with mesoderm on each side. A cross-section through the anterior end of the medullary plate is shown in Fig. XLVII. The thick plate of ectoderm is the medullary plate with a projecting median structure. The ectoderm extends a short distance to the left, and just beyond it a deep ingrowth of yolk cells makes the anal invagination, which lies not quite half way around the black border. The posterior tail-like part, seen in surface view, is probably a regenerative extension of the medullary folds.

The Immediate Effect of the Centrifugal Force.

In most cases a few of the eggs were preserved at the time they were taken from the wheel. Some of these were cut into sections. The condition of the eggs, from which the embryos described in Fig. 1 to Fig. 47 came, is illustrated in Fig. XL. It may be recalled that the eggs had been put on the machine at about the 128-cell stage, and had revolved at the rate of 180 revolutions per minute for $7^1/_2$ hours. The figure shows that the cleavage has continued in the upper hemisphere where many small cells are present. Cells have been formed even under the floor of the segmentation cavity. The egg appears not to have produced any more cleavage planes in the lower hemisphere than were present when it was put on the machine.

The eggs from which the embryos described as No. 48 to No. 51 developed, were put on the machine at the two-cell stage, or at the beginning of the cleavage period. Some of the eggs, when taken from the machine, were very much injured. It is probable that these, when put on the machine, had not developed so far as the others that are less injured. Only a few cells appear to have formed, Fig. XLI, which lie on one side of the egg at or below the equator. It can not be stated whether these cells appear on that side of the egg at which the embryo forms, for, however attractive such an hypothesis would be, there are no conclusive facts either for or against it; except that in other eggs in which an embryo appears it develops at about this level, and the other side of the egg appears undeveloped. Another egg of this same sort, but in a later stage, is shown in Fig. XXXVIII. The upper hemisphere is composed of a broken down mass of vacuolated substance with scattered pigment-blotches. The nuclei are swollen or else have disintegrated. Over a small part of one side of the egg — the upper left hand corner in the figure — a plate of small cells is present. It is the only trace of any definite structure in the egg.

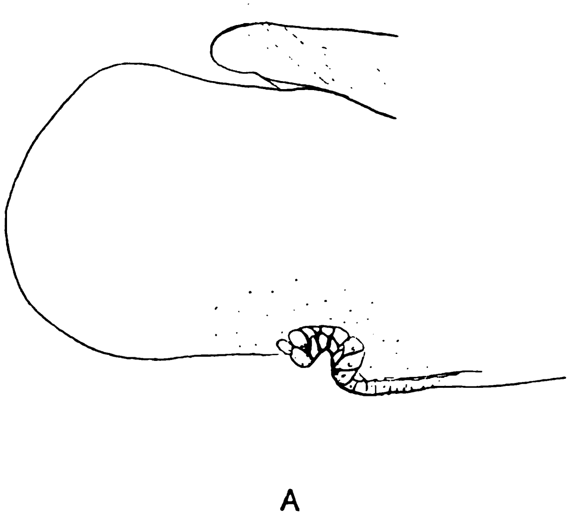
The other kinds of eggs in this lot are like that shown in Fig. XXXIX. A distinct segmentation cavity is present, and the upper hemisphere is divided into cells. The nuclei are confined to the region above the equator. The lower hemisphere is divided by a few vertical furrows. The egg is not very dissimilar from that of Fig. XL.

Finally I have made a few sections of eggs put on the machine before the cleavage had begun, and which did not subsequently divide. There is a watery zone or disc at the top of the egg. Below this there is a zone of finely granular material, which contains the pigment that has been driven out of the top of the egg. The rest of the egg is filled with yolk. No nuclei were found. In this case the influence of the centrifugal force has been so strong and the egg has been so much changed that it has failed to divide, and does not develop. It is interesting to note that these eggs were not rotated any faster than others that continued to segment. They were unsegmented when put on the wheel, and as the results show the unsegmented eggs can not withstand the influence of the centrifugal force so well as can eggs that have begun to segment when put on the wheel.

How, it may be asked, has the centrifugal force acted on the eggs so that they develop abnormally? In the first place, there can be no doubt that the centrifugal force tends to drive the heavier parts towards the white pole, and the lighter parts will rise to the black pole. The yolk granules seem to be heavier than the protoplasm in which they lie embedded, and we should expect them, in consequence, to be driven out of the upper hemisphere, and into that part of the egg that is turned outwards as it rotates. The sections show that a change of this sort does to some extent take place. But even if this is the case, will it account for the lack of development of the lower hemisphere? It seems to me that it is only one of the factors in the result. Another factor may be the failure of the nuclei to penetrate into the lower hemisphere. This may be due either to the nuclei which are lighter than the yolk being held in the lighter part of the egg, or, to the failure of the nuclei to penetrate into the denser parts of the egg. In addition to these two possibilities, that have been suggested by O. Hertwig, there may be a third and more important factor than either of these. Many different kinds of experiments have shown that the protoplasm of the white hemisphere is more easily injured than that in the upper parts of the egg, and the centrifugal force appears to have produced a similar effect and to have injured directly the protoplasm of the lower hemisphere, so that it fails to take part in the development.

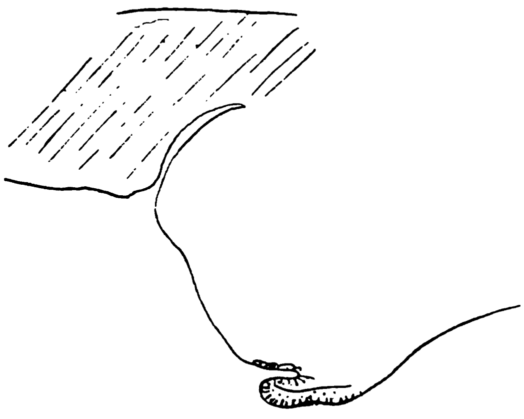
Older Less Injured Stages.

A not uncommon form of embryo found in later less injured stages is shown in Fig. 59. The yolk protrudes at the posterior end, and is surrounded by the thick lips of the blastopore. The blastoporic rim may be a continuation of a half-medullary fold, etc., on each side; or the median dorsal part of the embryo may, in some cases bend to one side only, and produce a whole structure along that border. In the latter case the other blastoporic rim would remain undeveloped, although it might later cover over the yolk at the side. In this embryo there is rather a larger posterior protrusion of yolk than in the average.



Still older stages of embryos that have been on the centrifugal machine show the condition represented in Figs. 60 and 61. At the posterior end there is a large plug of yolk. The black rim around the yolk represents, in most cases, the lips of the blastopore. In older stages the protruding yolk may still be present, but it finally disappears either by being drawn in, or sometimes by disintergrating. A large opening remains at the posterior end which seems to become the anus.

A longitudinal section showing the posterior end of the embryo drawn in Fig. 60 is drawn in Textfig. *A*. On the dorsal side, the archenteron opens to the exterior above the protruding yolk. On the ventral side there is an inturning of cells at the edge of the ectoderm. This is the anal invagination of earlier stages. A longitudinal median section of an older embryo, Textfig. *B*, shows at the posterior end the same conditions as before. The anal invagination is deeper in this case.



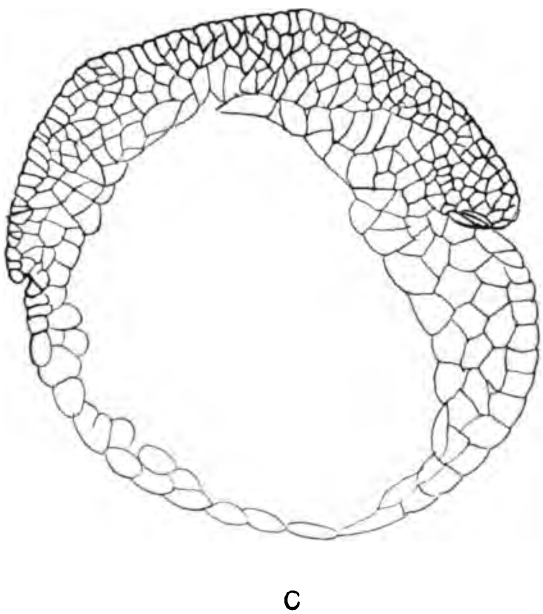
B

Effect of Cold on the Development.

A number of eggs of Rana palustris were put into the ice-chest just as the dorsal lip of the blastopore had appeared on many of them. They were left there for many days. During this time abnormal changes took place in the eggs. They became much larger than the normal egg, and a large fluid cavity could be seen in the yolk. The blastoporic lips extended around the egg along the line between the dark and light cells, or even higher up. Some of the embryos appeared as shown in Figs. 48 and 49. Sections of these eggs, Textfig. *C*, show that along the blastoporic line cells have pulled in to form the beginning of the archenteron. This is deeper on one side (anterior) of the egg than elsewhere. A large excentric cavity is present in the yolk. Whether this has been derived from the segmentation cavity can not be told. If so it has shifted its position. The most remarkable fact shown by the sections is the relatively large size of the cells of the upper hemisphere. This may be due in part to the absorption of water by the individual cells, but even if this is the case the cells are fewer in number than in a normal egg beginning to gastrulate. I conclude, therefore, that these eggs had not reached the gastrula-stage when put on the ice, and without dividing further they proceeded to gastrulate. It will also be seen, both in surface view and in section, that the blastoporic rim has developed above the equator of the egg. It lies much higher up on the egg than does the normal blastopore.

Some of the eggs on the ice produced abnormal embryos. One of these is shown in Fig. 50. A nearly complete medullary plate lies parallel to the black-white edge. There can be no doubt that the complete dorsal side has formed along one side of the blastopore as is the case in some of the eggs on the centrifugal machine. Another embryo is shown in Fig. 51. Here a broad, open medullary plate is present, that projects posteriorly over the yolk. The embryo is similar to those represented in Figs. 20 and 22 from the centrifugal machine.

Another embryo, Fig. 52, is also represented by an anterior medullary plate. At the posterior end two projections — looking like tail-knobs — extend backwards, from the edges of the medullary fold, over the yolk. The embryo is similar to those obtained by Gurwitsch that were placed in a $^1/_{1000}$ strychnine solution, which he thought had a specific action on the egg. This embryo — and the preceding one also — is the result of the failure of the dorsal lip to grow backwards, or rather it is due to the failure of the lateral lips to continue the dorsal lip backwards. The embryo not turning to one, or to the other side, remains short. It has secondarily grown backwards over the yolk.



A number of other abnormalities were also obtained in this set, especially such as had a shortened medullary plate and a posterior yolk exposure. The figures will suffice to show the similarity of some of these embryos to those from the centrifugal machine.

Spina bifida.

As Roux first pointed out it is not uncommon to find ring-embryos — the extremest form of spina bifida — in lots of eggs that have not been subjected to any special treatment, and in the same lots many normal embryos may be present. I have observed that these ring-embryos are most often found in the middle of bunches, or on the lower side, where the supply of oxygen, or the excess of carbon dioxide, or perhaps other influences help to bring about the result[1]). I have obtained some very extreme forms of this modification, which are here figured, Figs. 53—56, since they have a direct bearing on the discussion as to the correspondence, or to the lack of correspondence of the blastoporic rim in the ring-embryos and the early stages of the normal embryo. One of these embryos is shown in its anterior aspect (dorsal lip) in Fig. 53, and in posterior view in Fig. 54.

A thick ring runs around the egg somewhat above the equator. In the mid-posterior end a depression marks the anal invagination. A longitudinal section of the embryo is drawn in Fig. XLII. This shows even more distinctly that the embryonic rim has formed above the equator of the egg. A curious and striking fact is the presence in this embryo of little holes and splits in the yolk near the anterior end. Each such hole or split has a border of pigment. It is as though the entoderm cells were drawing apart at various points to form archenteric cavities. In part this phenomenon may be due to the development of the liver diverticulum, but it has also, I believe, a profounder significance, and represents a tendency of the yolk-cells to pull in to form the anterior end of the archenteron. It is, of course, possible that the pigmented cells have drawn in at the anterior rim of the blastopore, and, in fact, there is a narrow pigmented split in this region, but many of the cavities appear quite separate from the others.

Another similar ring-embryo is represented in Fig. 55 (anterior end) and in Fig. 56 (posterior end). Sections of this embryo were exactly like the last. The archenteric cavity is represented by a pigmented split beginning at the anterior blastoporic rim. It appears to be continuous, and not separated into different splits as in the former case. At the posterior end of this embryo, as seen in surface view, a groove is present leading backwards over the ectoderm from the edge of the blastoporic rim. The groove appears to represent the groove that in the normal embryo appears behind the closed blastopore, and leads to the invagination which forms the permanent anus, but this interpretation is open to question. The theoretical bearing of these ring-embryos will be discussed later.

---

[1]) It is also probable that internal changes may produce the same result.

Embryos with Open Medullary-Folds.

HERTWIG ('98) has described certain abnormal frog embryos in which the medullary plate fails to roll in and whose floor is occupied by abnormal cells. He founds these abnormalities very common in eggs that had developed at an unusually high temperature. MORGAN ('95) had previously obtained similar embryos with the teleost egg by diluting the sea-water with an equivalent amount of spring water. In one of my sets of frog's eggs, which had developed in dishes in the laboratory, similar embryos were found. The eggs had received no special treatment. Two of these are shown in Figs. 57 and 58. In the first, the blastopore has closed, but the medullary plate is still widely open, and its floor contains projecting groups of abnormal cells. The other embryo, Fig. 58, has the blastopore open and filled with the yolk. It is in this respect a typical spina bifida embryo. The anterior medullary plate has not closed in and its floor contains abnormal cells. Since most of the embryos in this bunch went normally, it is difficult to say what conditions brought about the result.

General Discussion of Results.

The interpretation of some of the abnormal embryos described in the preceding pages has been already implied in some of the brief descriptions, but we may make here a systematic examination of the results.

Half-Embryos.

Examples Figs. 5, 8, 37.

It is not infrequent to find half-embryos which are as strictly half as were those obtained first by ROUX, and later by others, when one of the first two blastomeres is killed. In both cases the anterior end, or head of the embryo is more than half, but in the middle and posterior regions of the body there is present a half-medullary plate, a half-archenteron, and the dorsal mesoderm is entirely, or, for the most part, on one side. In both cases the notochord is rounded, but apparently of smaller size than in the whole embryo. My results leave no doubt as to how these half-embryos are produced. The dorsal lip of the blastopore first appears, and its lateral lips begin to extend out on each side. Sometimes, the lateral lip extends out only on one side, and not at all, or to a limited extent, on the other. Wherever it extends the cells draw in along its path to form the archenteron. It is along this line that the half-embryo is laid down.

The anterior end of the embryo is formed from the continuous material in front of the dorsal lip, hence it is, as a rule, more or less complete. Along the edge of the blastopore the half-embryo is formed in much the same way as in cases of spina bifida, except that only one-half develops. The lack of development of the other half can be traced in all cases to the fact that this side of the egg is so injured that the lateral lip is not formed, or formed only near the dorsal lip. In proportion as both are laid down the head may extend further backwards, or an approach to spina bifida is made. It must be recalled that the dorsal and lateral lips are formed just below the segmentation cavity, and sections of early stages show that the material is often injured in this region. It is not surprising to find that occasionally it may be more injured on one side than on the other.

Whole Embryos along One Side of the Blastoporic Rim.

Examples Figs. 1, 4, 10, 17, 19, (27), 33, (63).

So many cases of this sort have been found that it is possible to state with some assurance how the result has come about. Moreover it is a result that has an important bearing on the principal question with which we have to deal. The embryo, drawn in Fig. 1, is a good example of this sort of development. The head stands nearly at right angles to the rim (or at least to that part of it that lies to the right), but the rest of the medullary-plate turns abruptly behind the head to run along the black edge. The all-important question to determine is whether the edge is the blastoporic rim of one side, or a new formation, having no relation to the normal rim. This embryo is too old to determine this point definitely, but several others leave no doubt on this point. The embryo drawn in Fig. 21, with its cross-section in Fig. XIX, shows clearly that the archenteric invagination, which is the most important point for the determination of the blastoporic rim, extends along the side of the medullary plate. This plate is not, to be sure, complete on the yolk side, but nevertheless it is more than half of a plate.

Another embryo, Fig. 19, is a lateral embryo that has a medullary fold also on the yolk-side, and the cross-section, Fig. XVII, shows that along the rim an archenteric invagination has taken place and still communicates with the exterior. The notochord is not immediately at the edge, but somewhat to the left (in the section). In this same section it is interesting to note that the ectoderm extends over only a part of the roof of the segmentation cavity, and on the side opposite that of the embryo there is no development of cellular material.

The impossibility of the extension of the other side of the blastopore into this region is apparent. This suggests immediately the idea that the formation of the whole embryo along one side is connected with the absence of the other half of the blastoporic rim. In fact the results establish this point of view with some degree of certainty.

Another embryo of the same kind is even more convincing, Figs. 33 and 34*A*. For its entire length the embryo lies along the dark edge. The sections show an archenteric opening along this edge. The notochord is some little distance from the edge and lies in the mid-dorsal line. The dorsal mesoderm is present on both sides of the notochord. It will be seen that in this case also the organized part lies only on one side of the segmentation cavity, the other not being cellular.

These cases will suffice to illustrate the main points although there are others, noticeably, Figs. 17, 29, 37, 7, which show the same conditions.

The point of great theoretical interest that is illustrated by these embryos is that a whole structure is formed along one side of the archenteric invagination. Out of material that under usual conditions would develop a half-structure, sometimes more than half is formed. The point that I wish especially to emphazise is not so much that a whole embryo forms out of material that would ordinarily make only a half-embryo¹), but rather that a structure (the blastoporic rim) is made use of, or is followed as a basis for the whole structure.

---

¹) I do not mean a preformed region in the egg, but that material, whatever its source, that lies along the blastoporic rim wherever it may appear on the egg.

The whole does not form anywhere in the organized material, but follows already existing lines.

The question of lateral regeneration, or post-generation, as ROUX calls it, may be raised here. Is a half-embryo first formed, as we may suppose ROUX would claim, and the other half postgenerated suddenly from it? Decidedly not, for while it is possible that such a method of completion does take place at times, and might make more complete the part laid down, yet this point of view is too narrow to explain all cases, or even most of them. At the time when ROUX formulated his view of post-generation many facts, which are now known, were unknown, or not fully appreciated. Later discoveries have shown that a whole structure may suddenly, as it were, be formed out of material already pre-arranged to form a part of a whole, but there can be little question that a certain amount of time is necessary to bring about this result, and that it is regulated by the heterotropic conditions in the part. This conception we must also apply to these cases of lateral whole development of the frog's egg.

The condition found in the youngest stage of the lateral whole embryo make it certain that the whole medullary plate is often laid down at once, although it is sometimes less complete on the yolk side than on the other, and sometimes in later stages the less complete side shows evidence of completing itself.

It has always been puzzling to me how a half-embryo could, as ROUX claims, post-generate the dorsal mesoderm on the incomplete side since the nervous system, and the notochord, which are laid down at the same time as the mesoderm, would apparently interfere with a migration of cells across the middle line. In the embryos that I have studied there is no difficulty in seeing how post-generation or regeneration of the missing mesoderm could take place, since in nearly every instance the notochord does not lie at the free edge, but somewhat inside of this, and a small cluster of mesoderm-cells is left on the open side of the embryo. From this as a blastema the missing part may be regenerated. Another possibility is that since the head is nearly bilateral there will be mesoderm in the anterior region on the undeveloped side of the embryo and this mesoderm might proliferate backwards, but there is no evidence that such a backgrowth takes place — or at least not to any great extent; and it is clear that the mesoderm of the middle and posterior end has had a different origin.

Anterior Embryos.

Examples Figs. 20, 22, 28, 32, 38.

In some cases only the anterior end of the embryo appears. This is due very often to a condition of spina bifida behind the anterior end, in which case the right and left halves of the posterior and middle parts of the embryo lie along the sides of the blastoporic rim. There is no difficulty in interpreting these cases, but there are others that are not so simple. For instance, in Fig. 32, the anterior end of the medullary plate is present, but at its posterior end it does not divide into half-plates extending around the blastoporic rim (although one side of the latter is thicker than the other). If the border represents an undeveloped blastoporic rim, as seems probably the case, it has failed to produce the characteristic structures. This we may ascribe to the material, where the lateral lip would have formed, being so injured that the archenteric invagination does not take place. The interpretation of this sort of embryo is then as follows. The dorsal lip appears, but extends laterally for only a very short distance, and since the lateral lips do not develop the embryo ends abruptly at the dorsal lip. In front of the dorsal lip the anterior end of the embryo forms on the undivided material. It may be that to some extent, the dorsal lip has grown over the surface of the yolk; and in proportion to its growth the part formed is longer, as in Fig. 32. In addition to this overgrowth, which is the same thing, in a way, as the normal overgrowth, there takes place in some cases a new kind of growth backwards, which is due to regeneration from the posterior end. A tail-like structure is the result, as shown in Figs. 43 and 65. This phenonenon is without parallel in the normal development and the process can not, I believe, be ascribed to a modification of any of the normal changes. We can not, for instance, infer that the result means that in the normal development some of the median material grows backwards along the mid-dorsal line; on the contrary, the normal development seems to show that this material is brought up from the sides.

Sections of these tail-like backgrowths show that along each side the ectoderm does not bend under to become continuous with the mesoderm as do the layers at the tail-knob of the selachian embryo. Such comparisons, like others of its kind, are, I think, entirely worthless.

In connection with these anterior embryos it should be pointed out that posterior embryos have never appeared in the material which I have examined. Roux doubtfully described embryos of this sort in some cases in which two of the first four blastomeres were killed. I have always been sceptical in regard to the possibility of such development if the anterior blastomeres have been killed. If Roux's hypothesis of mosaic development is true, then such a possibility may exist, but my own experience has lead me to the opinion that a posterior end does not develop unless there is an anterior end (or dorsal lip) at some region of the egg. I suspect the cases that Roux found were due to a retarded development of the anterior blastomeres.

In conclusion, our examination of the anterior embryos shows that they are produced by the lack of development of the lateral lips of the blastopore, just as we concluded in the last section that the lateral whole embryos are the result of the lack of development of one lateral lip of the blastopore.

Dorsal Embryos.

Examples Figs. 3, (27), (6).

It is doubtful if any of these embryos are, in a strict sense, dorsal embryos. The general appearence of Fig. 3 and the cross-sections, Figs. I, II, III, may seem to show that only the dorsal side of the embryo has developed. At the posterior end it is less developed on one side, as shown in Fig. III, which may mean that a blastoporic rim extended at first along this side and later closed over; but without more evidence I should not be willing to offer an interpretation of this case.

The other embryo, Fig. 27, is not so difficult to interpret. A plausible view can at least be offered as to its origin. It was at first a half-embryo, or more nearly a lateral whole embryo. The blastoporic rim extended along a part, at least of one side (the right). Its archenteric space was probably obliterated later by lateral growth of ectoderm over the side; as well as by the formation of ectoderm in the same region.

Another case is shown in Fig. 6. The sections through the middle and posterior of this embryo indicate, Fig. VII, that it also was at first a lateral whole embryo which has begun to close over its blastoporic side.

Ventral Embryos.

Examples Figs. 7, 7 *A*.

It is somewhat of an exaggeration to speak of this embryo as a ventral embryo, for although the whole of the ventral surface is present, yet not more so than in extreme cases of spina bifida. In fact this embryo appears to be a spina bifida embryo with something more than a half-embryo along one side of the blastoporic rim, as shown in Fig. X Plate XI. The other rim shows some indication of embryonic structures along it, but only to a limited extent. The surface view shows the entire ventral surface covered by ectoderm which forms near the anterior end a pair of suckers. The cross-section shows a continuous formation of mesoderm from the superficial yolk beneath the ectoderm.

Pieces of Embryos and Embryos of Reduced Size.

Examples Figs. 6, 12, 13, 14, 15, 30, 35, 36, 43, 45, 46, 62, 64, 65.

A number of the embryos are undoubtedly more or less reduced in size as compared with the normal. The fact that a large part of the yolk is injured and remains undeveloped does not in itself lead necessarily to the formation of a smaller embryo, since the yolk-mass in the normal embryo takes only a small part in the formation of the embryo[1]). On the other hand the uninjured protoplasm that produces the embryo is often less than the material out of which the normal embryo develops, and in consequence the size of the embryo is reduced. Examples of this are shown in Figs. 6, 14, 30. When the amount of material is further reduced only a part of an embryo may develop. The small half-embryo, shown in Fig. 5, is somewhat less than half of the whole, although nearly full length.

The embryo shown in Fig. 12 is the dorsal part of an embryo reduced in size. In Fig. 13 the embryo is little more than part of the anterior end. Somewhat larger are the embryos shown in Figs. 35 and 36, which represent reduced half-embryos. Finally the embryos in Figs. 45 and 46 are little more than pieces of the anterior ends of embryos. A point of importance in regard to all of these pieces of embryos is that in every case they have developed in connection with an archenteric invagination at the border of the dark area. It is, perhaps, not going too far to claim that without an archenteric ingrowth no part of the embryo of the frog can develop.

---

[1]) Meaning the dorsal part of the embryo in a strict sense.

This invagination is the starting point alike for the formation of an embryo, or for a part of one.

Blastoporic Rim.

Incidentally in the course of the description of the different embryos this topic has been discussed, so that a brief treatment must suffice here. It has been pointed out that the border between the ectoderm and the yolk does not always represent a blastoporic rim, as it does in the normal embryo when the sharp line between the ectoderm and yolk is established. A number of different conditions account for the free edge in the abnormal embryo.

In the first place, the ectoderm may develop only on one side of the egg and not over the other, or only over the roof of the segmentation cavity, as in the case of Figs. XVII, XVIII, XXX, and XXXI. Since in these cases there is no formation of cells on the side of the segmentation cavity opposite the embryo, there is no opportunity for the formation of an archenteron, even if the free edge of the ectoderm were prepared to take part in such a structure. At the posterior end of the embryo, or along its side, if it be a half-embryo, the archenteric invagination will take place. Its extension will be limited by the extent of the cellulated, organizable material of that side of the embryo. Beyond that point the free edge has no structural value. In other cases the free edge appears to represent a blastoporic rim, that has only incompletely developed, owing very probably to the abnormal condition of the material. It may in later stages succeed in completing itself so far as to take part in the final closing of the blastopore. A sort of incomplete (since there is scarcely any archenteric formation) reorganization takes place.

In a third category of cases the ectoderm along the dorsal part of a half-embryo, may, after the nervous system has formed, begin to extend over the yolk, as in one of the methods of re-organization described by Roux. In this case there is not, of course, any comparison at all to be made with a blastoporic rim.

In another category still, a different kind of edge is left, viz., in those cases in which the posterior end of the embryo grows backwards (regenerates) over the yolk, as shown in Figs. 32 and 43. In this case while the posterior end is, in the first instance, a blastoporic rim it is doubtful if the same interpretation can be applied to the sides of the backgrowth. There is certainly nothing in the structure to suggest such a comparison.

The value of the recognition of the different ways in which the ectoderm may end is negative, but may still be of service in guarding us against unwarrantable comparisons between the normal and the abnormal forms, and against the greater danger of comparing embryos of this sort with the embryos of other groups of vertebrates.

Anal Invagination.

In nearly all of the abnormal embryos a deeply pigmented pit is present at some point near the black border. I have called this the anal invagination without raising the question as to whether it represents the inturning that forms the middle of the posterior (or ventral) blastopore, or the invagination that becomes the permanent anus of the frog. In the normal development of the frog's embryo the posterior lip of the blastopore (which has grown forward by this time) is over-arched by the medullary folds and becomes the neurenteric canal. The anus is formed by a later ectodermal invagination just behind the neurenteric canal and outside of the medullary canal, as shown by GOETTE ('75), SCHULTZE ('84), SCHANZ ('87) and MORGAN ('89). Which of these two openings is the anal invagination? Does the posterior edge of the blastopore, or does the anus correspond to the invagination in these abnormal embryos? If the former does the permanent anus appears later? There can be no doubt that the pit corresponds to part of the blastoporic rim, because, in the first place, it is distinctly formed in the yolk-cells, and not in the ectoderm. Secondly, the cells that pull in become pigmented on the outer end as do those forming the edge of the blastopore; and thirdly, when a continuous blastoporic rim is present it is continuous with this invagination. In older embryos I have not found a secondary invagination forming the anus, but the large opening, at first filled with yolk, remains as the permanent anus, textfigs. *A*, *B*. It should be added, however, that I have not made a very thorough examination by means of sections of this point. If my conclusion is correct, the permanent anus is in many of these abnormal embryos somewhat different from that in the normal embryo. The point of special interest in these abnormal cases is the persistency with which this anal pit appears, even when there is scarcely a trace of the lateral lips of the blastopore. Moreover its relation to the posterior end of the embryo is very curious, yet can generally be accounted for. In an embryo, for instance, like that shown in Fig. 25, the anal pit lies on that part of the blastoporic rim that is nearly opposite the posterior end of the embryo. Should the yolk be drawn in later, and the black edge come together, the anal pit will be brought near the posterior end of the embryo. It lies, therefore, morphologically, in its proper position, provided we consider the black edge as the blastoporic rim. Most of the cases already described can be accounted for in this way. The only difficulty arises in those cases in which the black edge represents only in part a blastoporic rim. The apparent erratic location of the blastopore can be explained by its development at the farthest point (from the posterior end of the embryo) at which the edge is organized as a part of the embryo. In a case like that shown in Fig. 64*A*, where the anal pit lies at the farthest point of the black edge (to the left in the figure) we must look upon the rest of the edge, seen in the figure, as the blastoporic rim. Whether the other black edge is also an organized blastoporic rim makes little difference, since one organized lateral lip will suffice to locate the anal pit, as shown in the case of half-embryos. Whether in such cases the pit is a half-structure (in the sense that the rounded notochord is a half structure) can not be easily determined.

The position of the anal invagination in the embryo represented in Fig. 43, on the side of the egg opposite to that shown in the figure (at the level of the lower black tip) is an extreme case, and since the tail-like posterior end is a regenerated product its tip has no morphological relation to the anal pit.

The region of the anal pit is always connected with the rest of the embryo by organized material and is never found isolated from the rest[1]. The most striking fact is that this pit is invaginated even where there is no invagination along the lateral lips that lead to this region. A certain degree of autonomy in the formation of the anal pit must be admitted.

---

[1] Figs. 44 and 44*A* may appear to be an exception, but even here some sort of organized continuity between the two sides is probably present.

Abnormal Embryos of the Frog in Relation to the Specific Action of Modifying Influences.

In what sense, it may be asked, has the centrifugal force a specific action? Does it have a directive influence on the developing egg, or only a destructive influence on certain parts? The question may be put in another way, viz., is the effect the same as that obtained by mechanically injuring, by sticking for instance, localized areas. The answer is, I believe, that the centrifugal influence is essentially the same, but much more delicate since the egg as a whole is left intact.

If the action were a specific one, in a strict sense, we should expect to find a more definite type of abnormal development, while we find on the contrary an immense variety of forms. Nearly all of these abnormalities can, however, be reduced to a few simple modifications of the normal type of development, as the preceding pages have attempted to show. The differences can be referred to the extent of injury to the equatorial zone of the egg.

This point of view is supported by a comparison of the abnormalities described in the present paper with those that have been given by a number of workers, who have brought different influences to bear on the egg. The similarities between many of the embryos, especially the spina bifida forms, is very striking. It has been pointed out, in reviewing the literature, that the same kind of abnormalities have been produced by such diverce influences as several different salts, and poisons, by lack of oxygen, by too high and also by too low a temperature, by sticking the egg in different parts, etc. How can we explain this? Most simply I believe by supposing in all cases the same part of the egg has been injured so that it takes no active part in the development. The agents have been chosen of a kind and strength to injure the yolk part of the egg, and not, or to a less degree, the equatorial zone which contains the material out of which the embryo may form.

Under these conditions it is not surprising that the same or similar results are obtained. This brings us to a consideration of the equatorial part of the egg.

The equatorial zone is to be thought of as a superficial band around the egg, extending some distance on each side of the equator, and in eggs with an oblique axis standing also obliquely. Along its lower side the blastoporic rim appears, although, as some of the spina bifida embryos show, this rim may appear at any level of the equatorial zone.

The results reviewed in the first part of this paper have shown that one side of this zone is different from the rest, and this is the region in which the anterior end of the embryo appears; the dorsal lip of the blastopore appearing at its posterior border. Whether the material of the ring is totipotent need not be discussed, it will suffice if we can show that it is probably not isotropic but heterotropic.

The results described here as to the effects of the centrifugal force show, in practically all cases, that one region of the egg is less injured than the rest and in this region the dorsal lip of the blastopores appears. Two possibilities exist. First does the blastopore appear in the middle of the least injured part of the ring, or second, does it appear in the region at which normally the anterior end of the embryo develops and, hence, on the same part of the egg as does the normal embryo. The latter alternative seems to me much more probable, although the possibility of the other view must be freely granted. My reasons for adopting the one view as more probable are briefly these. The early stages of much injured eggs (Fig. XXVIII) show that cells develop only on one side of the ring (the region below the edge of the segmentation cavity). It seems to me improbable that this relation would be so constant did it not rest on some internal differences in the parts of the egg. Moreover the dorsal lip of the blastopore does not always appear symmetrically in the middle of a developing region, but at times quite to one side of it, as when half-embryos, or lateral whole embryos are formed. This would seem to indicate that the dorsal lip appears on a definite part of the egg.

It is more doubtful whether the following fact has an important bearing on this point. If it be granted that the egg is so organized that the dorsal lip appears in a definite region determined by the presence of some material, or by the presence of some structural organization, then when the egg becomes divided into cells the opportunity for re-arrangement would be less marked than before division of the egg. Yet some of the best material has come from eggs that were in the 128-cell stage when put on the machine.

Finally it must be admitted, that in the most extreme cases where the action of the centrifugal force has gone so far that only a piece of an embryo, or an extremely small embryo appears on the egg, it is very difficult to show that the re-organization of the less injured part has taken place along the old lines. But if we can show that such a directive influence is present in nearly all other cases we need not be disturbed by the difficulty of explaining the most extreme forms of abnormalities, since the probability is that they are not exceptions to the rule. If we can show that a simple hypothesis will explain the great majority of cases and that those to which we have difficulty in applying the same explanation are too complicated to follow we can safely leave them to future investigation.

We stated at the outset that our main object was to determine whether the abnormal development takes place along the lines of the original organization, and according to the same general type of development, although not necessarily in either case out of the same material, part for part.

We have tried to show that the egg is not isotropic, but heterotropic in structure; that after injury, or disturbance the re-organization is along the old lines of structure, and that while the material may be in large part totipotent, nevertheless the reconstruction is along already existing lines.

Summary.

1) Eggs of Rana palustris in the 2, 4, 128-cell stage were revolved on a wheel turning at the rate of 160—180 revolutions per minute. The eggs were taken from the wheel after 8 to 10 hours and continued to develop, producing a great variety of forms.

2) The immediate effect of the rotation is to drive more yolk into the white hemisphere. The failure of the nuclei to penetrate into the white hemisphere accounts also in part for the formation of the abnormal development. The most important effect, however, of a strong centrifugal force is the direct injury to the protoplasm of the lower hemisphere of the egg.

3) Some of the eggs produce half-embryos, Figs. 5, 8, 37, which resemble those obtained by killing one of the two first blastomeres in Roux's experiment. The explanation of this result is to be found in the greater injury to one half of the egg than to the other and to the failure of the missing half to develop on the more injured side.

4) Whole embryos of half size sometimes develop, Figs. 1, 4, 10, 17, 19, 33. They are formed along one side of the blastoporic rim, where an invagination takes place. The presence of a half structure

(the blastoporic invagination) is made use of in the formation of a whole embryo. The greater injury (than in the last case) of the other undeveloped side determines probably that a whole rather than a half development takes place.

5) The anterior part of an embryo is sometimes formed, Figs. 20, 22, 28, 32, 38. This is due to the formation of only the dorsal lip of the blastopore, the lateral lips failing to develop because of the injury to the rest of the egg. In front of the dorsal lip the anterior end of the embryo develops. Secondarily a kind of regeneration may take place from the posterior end of the anterior embryo, Figs. 47, 65.

6) A sort of dorsal embryo appears in a few cases, Figs. 3, 27. It is formed along a blastoporic rim and may be said to be something more than a half-dorsal embryo.

7) Ventral embryos, like the one shown in Figs. 7, 7*A*, are really only extreme cases of spina bifida with widely open dorsal surface.

8) Pieces of embryos sometimes appear, Figs. 6, 17, when only a small part of the egg remains alive. These pieces correspond to small dorsal and anterior pieces of embryos.

9) The blastoporic rim is not always coextensive with the black white border. The latter may represent a number of different conditions as explained on page 301.

10) A marked independence of the invagination in the middle part of the ventral lip (the anal pit) is observed in many of the embryos, but it appears only when some part of the more anterior regions of the embryo are present.

11) The centrifugal force may be said to have a specific action only in the sense that it renders certain parts of the egg incapable of development. The great variety of forms that arise are the result of the extent of injury to different parts of the egg.

12) If eggs of Rana palustris, that have segmented a number of times, are kept at about the temperature of 0° C. the further cleavage is retarded. In extreme cases a ring-like archenteric invagination may take place above the equator of the egg. The invagination may be formed in eggs in which the cells are still much larger than in the normal egg at the time of gastrulation.

13) Extreme cases of spina bifida that produce ring-embryos, Figs. 53—54, 55—56, show that the archenteric invagination may take place above the equator of the egg. This result demonstrates that while the abnormal embryo may follow the same type of develop-

ment as the normal it need not necessarily develop out of the same material of the egg.

14) The general conclusion to be deduced from the preceding results is that while the material of the egg may be totipotent, yet the protoplasm is heterotropic; and in cases of abnormal development the development proceeds along the lines of structure which are followed by the normal embryo.

Zusammenfassung.

1) Eier von Rana palustris im 2-, 4-, 128 Zellen-Stadium wurden auf einem Rade mit umgedreht, welches 160—180 Umdrehungen pro Minute machte. Die Eier wurden nach 8—10 Stunden von dem Rade genommen und setzten ihre Entwickelung weiter fort, wobei eine große Verschiedenheit der Formen entstand.

2) Der unmittelbare Effekt der Rotation ist der, dass mehr Dotter in die weiße Hemisphäre getrieben wird. Die Unfähigkeit der Kerne, in die weiße Hemisphäre einzudringen, ist zum Theil an dem Eintritt abnormaler Entwickelung schuld. Der wichtigste Effekt einer starken Centrifugalkraft ist jedoch die direkte Schädigung des Protoplasmas der unteren Eihälfte.

3) Manche Eier ergeben Hemiembryonen, Fig. 5, 8, 37, welche denen ähneln, die man beim Roux'schen Versuch durch Tödtung einer der beiden ersten Blastomeren erhält. Die Erklärung dieses Ergebnisses ist in größerer Schädigung einer Eihälfte gegenüber der anderen zu suchen, und in der Unfähigkeit der übrig bleibenden, die Entwickelung auf die stärker geschädigte Seite hinüber fortzusetzen.

4) Ganze Embryonen von halber Größe entwickeln sich auch manchmal, Fig. 1, 4, 10, 17, 19, 33. Sie entwickeln sich entlang einer Seite der Blastoporusspalte, wo eine Einstülpung stattfindet. Die schon vorhandene Halbbildung (die Blastoporuseinstülpung) wird bei der Bildung eines ganzen Embryos benutzt. Wahrscheinlich bestimmt die größere Schädigung (gegenüber obigem Fall) der anderen unentwickelten Seite, dass es eher zur Entwickelung eines ganzen, als eines halben Embryos kommt.

5) Bisweilen entwickelt sich nur der Vordertheil eines Embryo, Fig. 20, 22, 28, 32, 38. Das hängt von der alleinigen Entwickelung der dorsalen Blastoporuslippe ab, während die seitlichen Lippen der Schädigung des übrigen Eitheils wegen sich nicht entwickeln. Nach vorn von der dorsalen Lippe entwickelt sich das Vorderende des Embryos. Sekundär kann eine Art Regeneration stattfinden, welche von dem hinteren Ende des Vorderhemiembryos ausgeht (Fig. 47, 65).

6) In manchen Fällen erscheint eine Art dorsaler Embryo, Fig. 3, 27. Er bildet sich längs einer Blastoporusspalte und kann als etwas mehr als ein dorsaler Hemiembryo aufgefasst werden.

7) Ventrale Embryos, wie die in Fig. 7, 7 *A*, sind in Wirklichkeit lediglich extreme Fälle von Spina bifida mit weit offener Rückenfläche.

8) Stücke von Embryonen erscheinen manchmal, Fig. 6, 17, wenn nur ein kleiner Theil des Eies am Leben bleibt. Diese Stücke entsprechen kleinen dorsalen und vorderen Stücken von Embryonen.

9) Die Blastoporusspalte richtet sich in ihrer Ausdehnung nicht immer nach der Grenze zwischen schwarz und weiß. Die letztere hängt von einer Anzahl verschiedener Bedingungen ab, welche auf pag. 301 erörtert werden.

10) Bei manchen Embryonen zeigt die Einstülpung im mittleren Theil der ventralen Lippe (das Analgrübchen) eine deutlich ausgesprochene Unabhängigkeit; es erscheint jedoch nur, wenn ein gewisser Theil der mehr nach vorn gelegenen Abschnitte des Embryos vorhanden ist.

11) Man kann der Centrifugalkraft nur in dem Sinne eine specifische Wirkungsweise zuschreiben, dass sie gewisse Theile des Eies zur Entwickelung unfähig macht. Die große Verschiedenheit der entstehenden Formen entspricht den verschiedenen Theilen des Eies, auf die sich die Störung erstreckt.

12) Wenn Eier von Rana palustris, welche sich eine Anzahl von Malen gefurcht haben, bei einer Temperatur von ungefähr 0° C. gehalten werden, so wird die weitere Furchung verzögert. In extremen Fällen kann es zu einer ringförmigen Einstülpung des Archenterons oberhalb des Eiäquators kommen. Die Einstülpung kann sich dabei an Eiern ausbilden, deren Zellen noch sehr viel größer sind, als die von normalen Eiern zur Zeit der Gastrulation.

13) Extreme Fälle von Spina bifida, welche zur Bildung von Ringembryonen führen, Fig. 53—54, 55—56, zeigen, dass die Archenteroneinstülpung oberhalb des Eiäquators stattfinden kann. Dieses Ergebnis zeigt, dass der abnorme Embryo, während er demselben Entwickelungstypus folgt wie der normale, nicht nothwendig sich aus demselben Eimaterial aufzubauen braucht wie dieser.

14) Das Hauptergebnis der vorstehenden Einzelresultate ist das, dass, während das Eimaterial totipotent sein kann, doch das Protoplasma heterotropisch ist, und dass in Fällen abnormer Entwickelung die Entwickelung gemäß den Strukturgrundzügen fortschreitet, nach denen sich auch der normale Embryo richtet.

Literature.

Assheton, R., On the Growth in length of the Frog Embryo. Quart. Journ. Micros. Science. XXXVII. 1895.

Barfurth, D., Halbbildung oder Ganzbildung von halber Größe. Anat. Anz. 1893.

—— Über organbildende Keimbezirke und künstliche Missbildungen des Amphibieneies. Anat. Hefte. III. 1894.

Bataillon, E., Etudes expérimentales sur l'Evolution des Amphibiens. Archiv f. Entwickelungsmech. XII. 1901.

—— Sur l'Evolution de la fonction respiratoire chez les oeufs d'Amphibiens. Ibid. XII. 1901.

—— La pression osmotique et les grands problèmes de la Biologie. Archiv f. Entwickelungsmech. XI. 1901.

—— Recherches anatomiques et experimentales sur la Métamorphose des Amphibiens anoures. Annales de l'Université de Lyon. II. 1891.

—— Nouvelles Recherches sur les Mécanismes de l'Evolution. Les premiers stades du developpement chez les Amphibien et les Poissons. Arch. de Zool. experim. V. 1897.

v. Bambeke, Ch., Nouvelles Recherches sur l'Embryologie des Batraceens. Arch. de Biol. I. 1880.

v. Bambeke, Ch., Le Sillon Médian ou Raphé gastrulaire du Triton alpestre. Arch. de Biol. XIII. 1893.

Born, E., Biologische Untersuchungen. I. Über den Einfluss der Schwere auf das Froschei. Arch. f. mikr. Anat. XXIV. 1885.

—— Über Druckversuche an Froscheiern. Anat. Anz. VIII. 1893.

—— Neue Kompressionsversuche an Froscheiern. Schles. Ges. f. vaterl. Kultur. LXXII. 1894.

Endres, H., Über Anstich- und Schnürversuche an Eiern von Triton taeniatus. Sitzber. d. schles. Ges. f. vaterl. Kultur. 1895.

—— Über Anstichversuche an Froscheiern. Jahresber. d. schles. Ges. f. vaterl. Kultur. 1894.

—— Anstichversuche an Eiern von Rana fusca. Erster Theil. Archiv f. Entwickelungsmechanik. II. 1896.

—— Zweiter Theil. Ergänzung durch Anstichversuche an Eiern von Rana esculenta, etc. Ibid.

v. Erlanger, R., Über den Blastoporus der anuren Amphibien. Zool. Jahresber. IV. 1890.

—— Zur Blastoporusfrage bei den anuren Amphibien. Anat. Anz. VI. 1891.

Eycleshymer, A. C., The Early development of Amblystoma with Observations on some other Vertebrates. Journ. of Morph. X. 1895.

—— The location of the Bases of the Amphibian Embryo. Journ. of Morph. XIV. 1898.

Godlewski, E., Über die Einwirkung des Sauerstoffs auf die Entwickelung und über den Gaswechsel in den ersten Entwickelungsstadien von Rana temporaria. Bull. Internat. de l'Academie des Sciences de Cracovie. July 1900.

—— Die Einwirkung des Sauerstoffes auf die Entwickelung von Rana temporaria. etc. Archiv f. Entwickelungsmech. XI. 1901.

Gurwitsch, Über die formative Wirkung des veränderten chemischen Mediums auf die embryonale Entwickelung. Archiv f. Entwickelungsmech. III. '96,

Hertwig, O., Die Entwickelung des Keimblattes der Wirbelthiere. Jen. Zeitschrift. XV. 1882. XVI. 1883.

—— Das Problem der Befruchtung und der Isotropie des Eies, eine Theorie der Vererbung. 1884.

—— Urmund und Spina bifida. Arch. f. mikr. Anat. XXXIX. 1892.

—— Über den Werth der ersten Furchungszellen für die Organbildung des Embryo. Arch. f. mikr. Anat. XLII. 1893.

—— Experimentelle Erzeugung thierischer Missbildungen. Festschr. zum 70. Geburtstage v. Carl Gegenbaur. II. 1896.

—— Beiträge zur experimentellen Morphologie und Entwickelungsgeschichte. Theil I. Die Entwickelung des Froscheies unter dem Einfluss schwächerer und stärkerer Kochsalzlösungen. Arch. f. mikr. Anat. XLIV. 1895.

—— Über den Einfluss äußerer Bedingungen auf die Entwickelung des Froscheies. Sitzber. d. k. Pr. Akad. d. Wiss. Phys.-math. Abth. 1894.

—— Über den Einfluss verschiedener Temperaturen auf die Entwickelung der Froscheier. Ibid. 1896.

—— Über einige am befruchteten Froschei durch Centrifugalkraft hervorgerufene Mechanomorphosen. Sitz. d. k. Pr. Akad. d. Wiss. 1897.

—— Über den Einfluss der Temperatur auf die Entwickelung von Rana fusca und Rana esculenta. Arch. f. mikr. Anat. LI. 1898.

Hertwig, O., Beiträge zur experimentellen Morphologie u. Entwickelungsgeschichte. 4. Über einige durch Centrifugalkraft in der Entwickelung des Froscheies hervorgerufene Veränderungen. Arch. f. mikr. Anat. LIII. 1898.

Houssay, F., Formation de la Gastrula du Mésoblast et de la Chorda dorsale chez l'Axolotl. Compt. Rend. CVII. 1888.

—— E'tudes d'Embryologie sur les Vertébrés. Arch. zool. expér. VIII. 1890.

Jordan, E. O., and Eycleshymer, A. C., The Cleavage of the Amphibian Ovum. Anat. Anz. VII. 1892.

—— —— On the Cleavage of Amphibian Ova. Journ. of Morph. IX. 1894.

Kathariner, L., Über die bedingte Unabhängigkeit der Entwickelung des polar differenzirten Eies von der Schwerkraft. Archiv f. Entwickelungsmech. XII. 1901.

Kopsch, Fr., Über das Verhältnis der embryonalen Achsen zu den drei ersten Furchungsebenen beim Frosch. Internat. Monatsschr. f. Anat. u. Physiol. XVII. 1900.

—— Über die Zellenbewegungen während des Gastrulationsprocesses an den Eiern von Axolotl und vom braunen Grasfrosch. Sitz. d. Ges. naturf. Freunde zu Berlin. 1895.

—— Beiträge zur Gastrulation beim Axolotl- und Froschei. Verh. Anat. Ges. Basel. 1895.

Loeb, J., Über die relative Empfindlichkeit von Fischembryonen gegen Sauerstoffmangel und Wasserentziehung in verschiedenen Entwickelungsstadien. Pflüger's Archiv. 55. '94.

—— Untersuchungen über die physiologischen Wirkungen des Sauerstoffmangels. Pflüger's Archiv. 62. '93.

Morgan, T. H., On the Amphibian Blastopore. Studies Biol. Lab. Johns Hopkins Univ. IV. 1889.

—— Some Notes on the Breeding Habits and Embryology of Frogs. American Naturalist. XXV. 1891.

—— The Formation of the Embryo of the Frog. Anat. Anz. IX. 1894.

—— Half-Embryos and whole Embryos from one of first two Blastomeres of the Frog's Egg. Anat. Anz. X. 1895.

—— The Development of the Frog's Egg. New York. 1897.

—— The Problem of Development. The International Monthly. March 1901.

Morgan, T. H., and Tsuda, Umé, The Orientation of the Frog's Egg. Quart. Journ. micr. Science. XXXV. 1894.

Pflüger, E., I. Abhandlung. Über den Einfluss der Schwerkraft auf die Theilung der Zellen. Pflüger's Archiv. XXXI. 1883.

—— II. Ibid. XXXII.

—— III. Über die Einwirkung der Schwerkraft auf die Theilung der Zellen. etc. Ibid. XXXIV. 1884.

Rauber, A., Primitivrinne und Urmund. Morph. Jahrb. II. 1876.

—— Neue Grundlegungen zur Kenntnis der Zelle. Morph. Jahrb. VIII. 1883.

—— Furchung und Achsenbildung bei Wirbelthieren. Zool. Anz. VI. 1883.

—— Schwerkraftversuche an Forelleneiern. Berichte d. naturforsch. Gesellsch. Leipzig. 1884.

—— Uber den Einfluss der Schwerkraft auf die Theilung und das Wachsthum. Ibid. 1884.

—— Furchung und Achsenbildung. Zool. Anz. IX. 1886.

ROUX, W., Über die Zeit der Bestimmung der Hauptrichtungen des Froschembryo. Leipzig. 1883.

—— Über die Entwickelung der Froscheier bei Aufhebung der richtenden Wirkung der Schwere. Breslauer ärztl. Zeitschr. 1884.

—— Zur Orientirung über einige Probleme der embryonalen Entwickelung. Zeitschr. f. Biologie. XXI. 1883.

—— Über die Bestimmung der Hauptrichtungen des Froschembryo im Ei und über die erste Theilung des Froscheies. Ibid. 1885.

—— Die Bestimmung der Medianebene des Froschembryo durch die Kopulationsrichtungen des Eikernes und des Spermakernes. Arch. f. mikr. Anat. XXXIX. 1887.

—— Über die künstliche Hervorbringung halber Embryonen durch Zerstörung einer der beiden ersten Furchungskugeln etc. VIRCHOW's Archiv. CXIV. 1888.

—— Über die Lagerung des Materials des Medullarrohres im gefurchten Froschei. Verh. der Anat. Gesellsch. Würzburg. 1888.

—— Zur Frage der Achsenbestimmung des Embryo im Froschei. Biol. Centralbl. VIII. 1888.

—— Über das entwickelungsmechanische Vermögen jedes der beiden ersten Furchungszellen des Eies. Verh. d. Anat. Gesellsch. Wien. 1892.

—— Über die Mosaikarbeit und neuere Entwickelungshypothesen. Anat. Hefte. MERKEL und BONNET. 1893.

—— Über die Specifikation der Furchungszellen und über die bei der Postgeneration und Regeneration anzunehmenden Vorgänge. Biol. Centralbl. XIII. 1893.

—— Über die ersten Theilungen des Froscheies und ihre Beziehung zu der Organbildung des Embryo. Anat. Anz. VIII. 1893.

—— Berichtigungen zu O. SCHULTZE's jüngstem Aufsatz über die Bedeutung der Schwerkraft etc. Archiv f. Entwickelungsmech. X. 1900.

—— Bemerkungen zu O. SCHULTZE's Arbeit über die Nothwendigkeit der »freien Entwickelung« des Embryo etc. Archiv f. Entwickelungsmech. IX. 1900.

—— Bemerkungen zu O. SCHULTZE's neuen Rotationsversuchen an Froscheiern. Archiv f. Entwickelungsmech. V. 1897.

ROBINSON, A., and ASSHETON, The Formation and Fate of the Primitive Streak, etc. Q. J. Micr. Sc. XXXII. 1891.

SAMASSA, H., Über die äußeren Entwickelungsbedingungen von Rana temporaria. Verh. d. deutsch. Gesellsch. '96.

—— Über die Einwirkung von Gasen auf die Protoplasmaströmung und Zelltheilungen von Tradescantia, sowie auf die Embryonalentwickelung von Rana und Ascaris. Verh. d. naturhist.-med. Vereins zu Heidelberg. N. F. VI. '98.

SCHANZ, Das Schicksal des Blastoporus bei den Amphibien. Jen. Zeitschr. XXIV. 1887.

SCHULTZE, O., Beitrag zur Entwickelungsgeschichte der Batrachier. Inaug. Diss. 1883.

—— Zur ersten Entwickelung des braunen Grasfrosches. Gratulationsschrift f. v. KÖLLIKER. 1887. (Review by ROUX in Biol. Centralbl. VII. 1887.)

—— Über die Entwickelung der Medullarplatte des Froscheies. Verh. der phys.-med. Gesellsch. zu Würzburg. XXIII. 1889.

SCHULTZE, O., Die künstliche Erzeugung von Doppelbildungen etc. Archiv f. Entwickelungsmech. I. 1894.

—— Über die Einwirkung niederer Temperatur auf die Entwickelung des Frosches. I. Anat. Anz. X. 1895.

—— Neue Untersuchungen über die Nothwendigkeit der richtenden Wirkung der Schwerkraft für die Entwickelung. Sitz. d. Ver. d. Phys.-med. Gesellschaft zu Würzburg. VII. 1897.

—— Neue Untersuchungen zur Frage von der Nothwendigkeit der Schwerkraft für die Entwickelung. Verh. d. anat. Gesellsch. zu Gent. 1897.

—— Über die Einwirkung niederer Temperatur auf die Entwickelung des Frosches. II. Anat. Anz. XVI. 1899.

—— Die bilaterale Symmetrie des Amphibieneies. Verh. d. anat. Gesellsch. zu Tübingen. 1899.

—— Über den Einfluss des Luftmangels auf die erste Entwickelung des Eies. Verh. d. phys.-med. Gesellsch. zu Würzburg. XXXII. 1899.

—— Über den Einfluss des Luftmangels auf die erste Entwickelung des Eies. Verh. d. phys.-med. Gesellsch. zu Würzburg. 1899.

—— Zur Frage von der Entwickelung der Doppelbildungen. Centralbl. f. allg. Pathol. und patholog. Anat. X. 1899.

—— Über Achsenbestimmung des Froschembryo. Biol. Centralbl. VII. 1887.

—— Die Entwickelung der Keimblätter und der Chorda dorsalis von Rana fusca. Zeitschr. f. wiss. Zool. XLVII. 1888.

—— Zur Frage von der Bedeutung der Schwerkraft für die Entwickelung des thierischen Embryo. Archiv f. mikr. Anat. LVI. 1900.

—— Über das erste Auftreten der bilateralen Symmetrie im Verlaufe der Entwickelung. Arch. f. mikr. Anat. LV. 1900.

—— Über die Nothwendigkeit der freien Entwickelung des Embryo. Arch. f. mikr. Anat. LV. 1900.

SCHWINK, F., Über die Gastrula bei Amphibieneiern. Biol. Centralbl. VIII. 1888.

WILSON, H. V., Formation of the Blastopore in the Frog's Egg. Anat. Anz. XVIII. 1900.

—— Closure of the Blastopore in the normally placed Frog's Egg. Anat. Anz. XX. 1901.

Description of Figures.

Plates VIII—XII.

For description of figures see text.

Figs. 1—46. From eggs that had been put on the centrifugal machine in the 128-cell stage and left $7^1/_2$ hours.

Figs. 48—52. Frog's eggs that had been kept in an ice-chest.

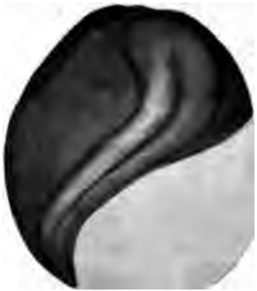
Figs. 53—56. »Ring-embryos« found in normal bunch of eggs.

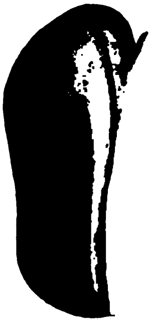
Figs. 57—58. Embryos with open (abnormal) medullary folds.

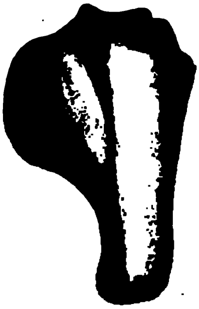
Figs. 59—61. Later stages of embryos from centrifugal machine.

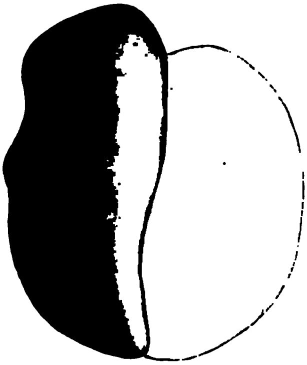
Figs. 47, 62—65. From eggs that had been put on the centrifugal machine at the 2-cell stage.

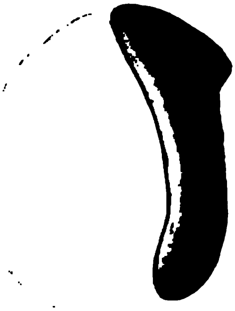
Figs. I to XLVII (Plates XI and XII). Sections of embryos (see text for description). Fig. XXIV is a part of Fig. XXV.

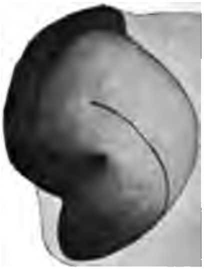

1

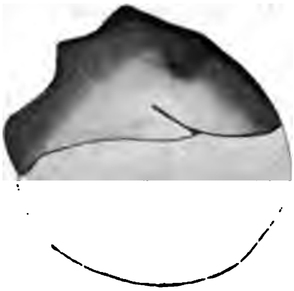

2

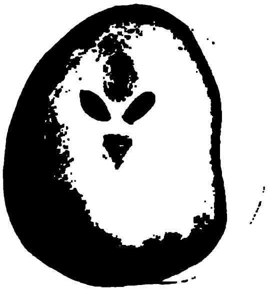

3

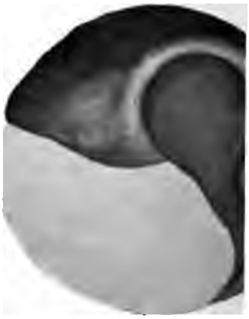

4

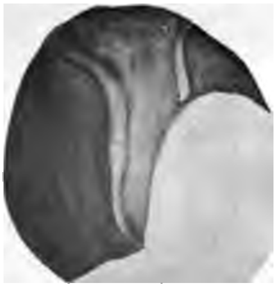

5

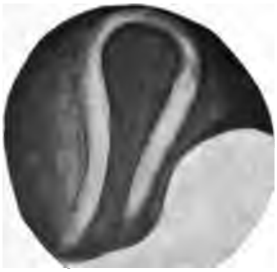

6

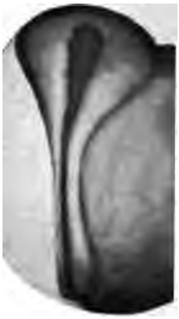

7

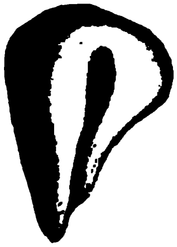

7A

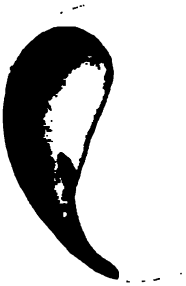

8

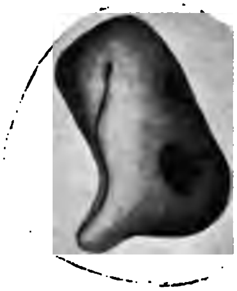

9

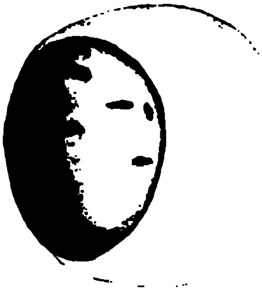

10

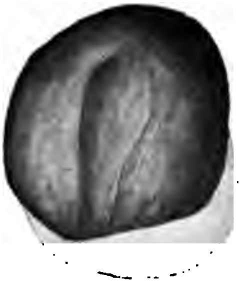

11

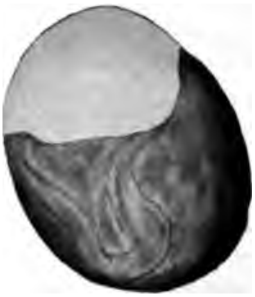

12

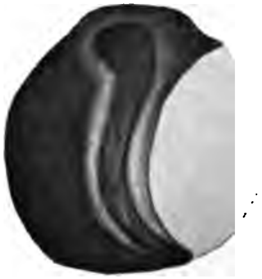

13

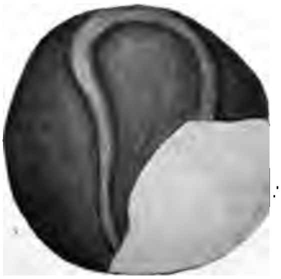

14

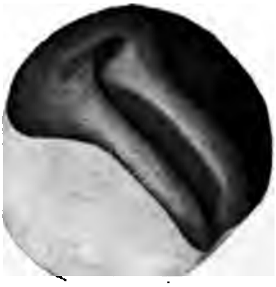

15

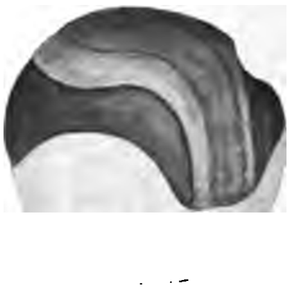

16

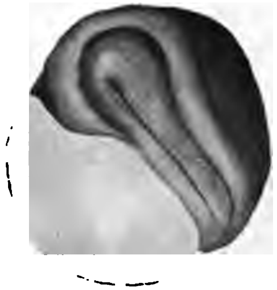

16A

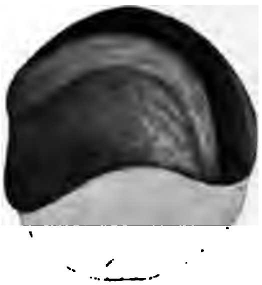

17


18

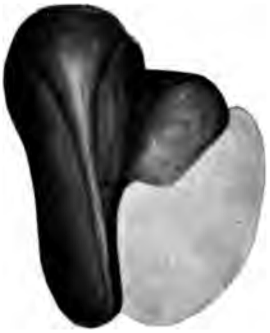

19


20

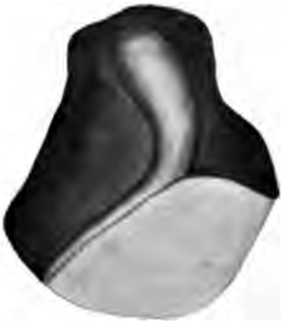

21


22

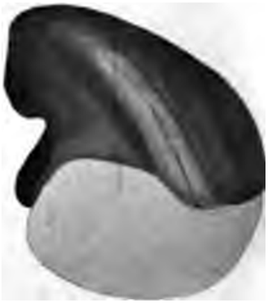
Verlag von Wilhelm Engelmann in Leip



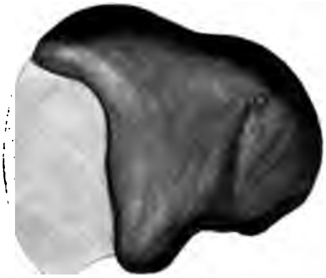
23



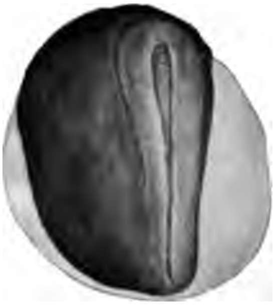
24



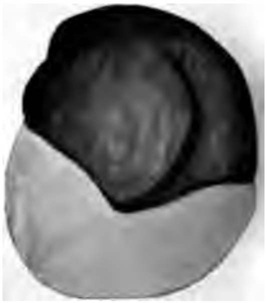
25



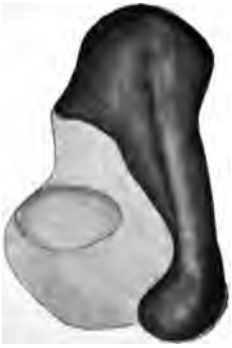
26



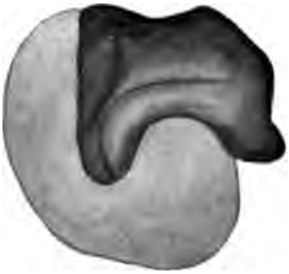
27



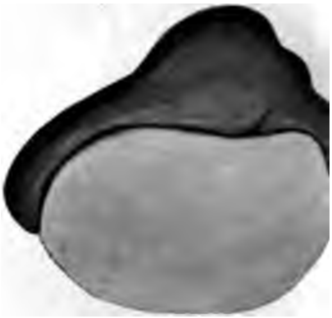
28



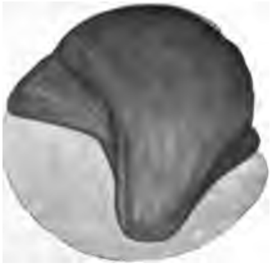
29



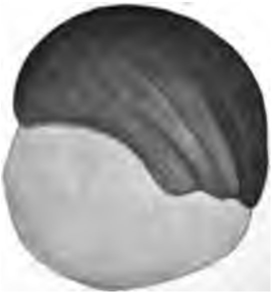
30



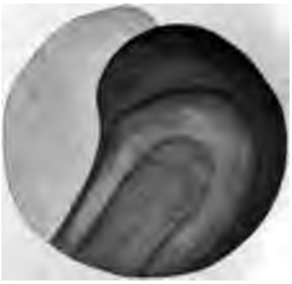
31



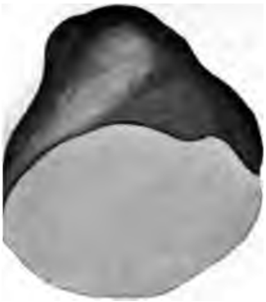
32



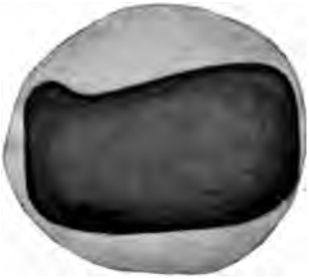
33



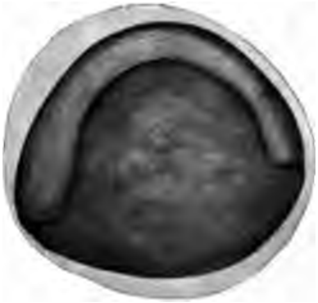
33 A



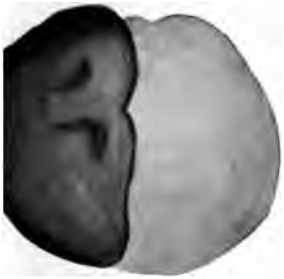
34



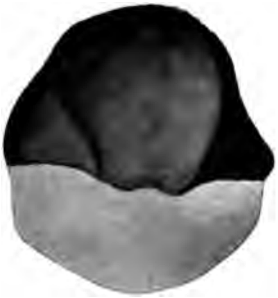
35



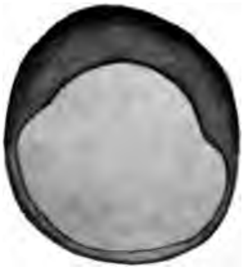
36



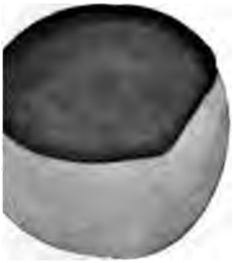
37



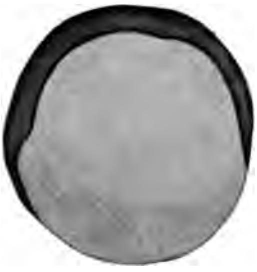
38



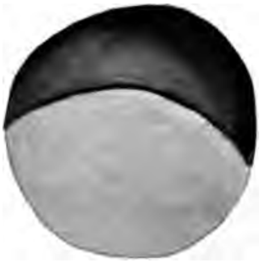
39



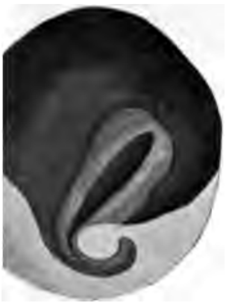
40



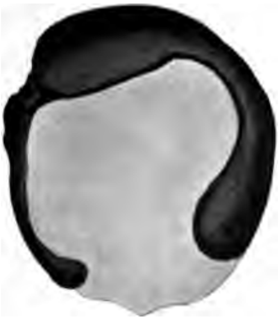
41



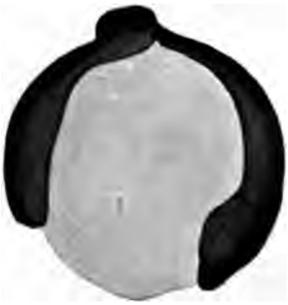
42



43



44



44A

Verlag von Wilhelm Engelmann in Leipzi

45

46

47

48

49

50

51

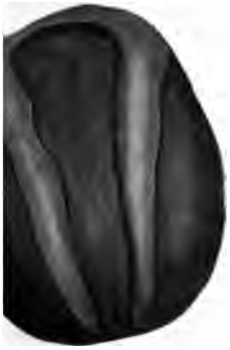
52

53

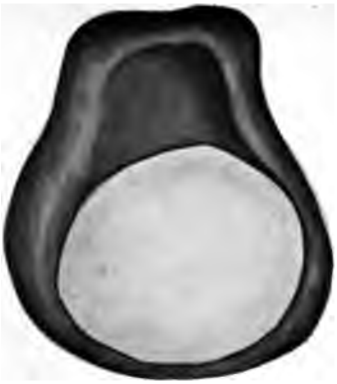
54

55

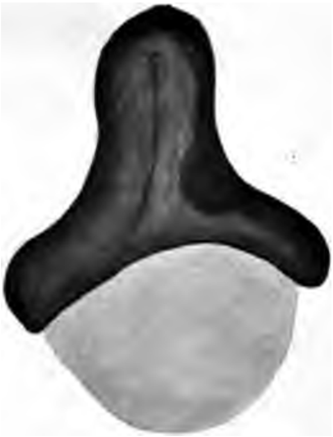
56



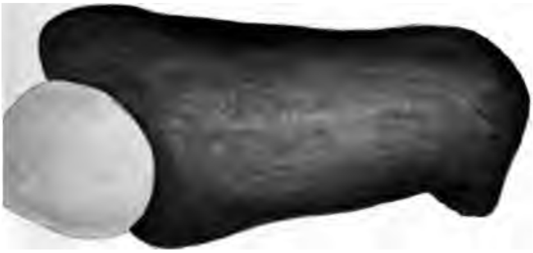
57



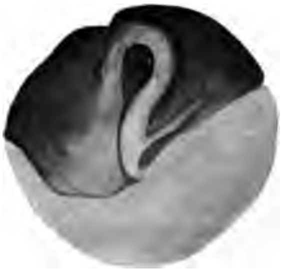
58



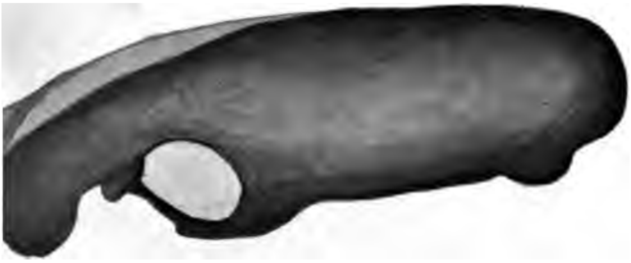
59



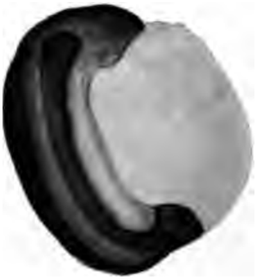
60



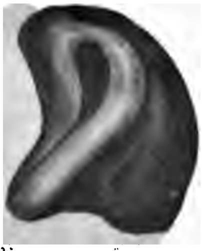
62



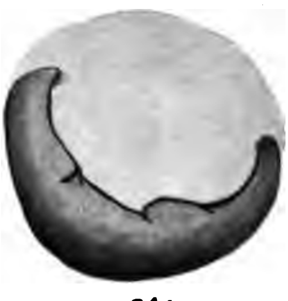
61



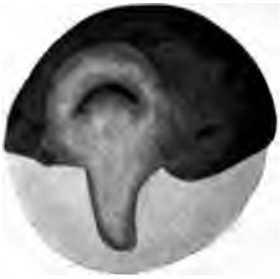
63



64

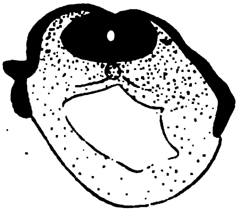


64A

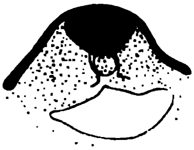


65

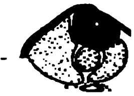
Verlag von Wilhelm Engelmann in Le



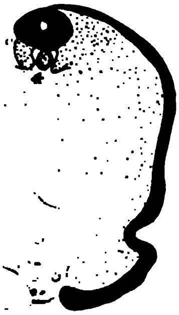
I



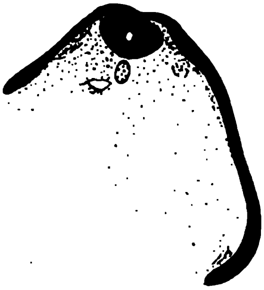
II



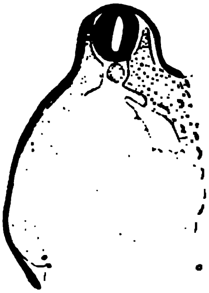
III



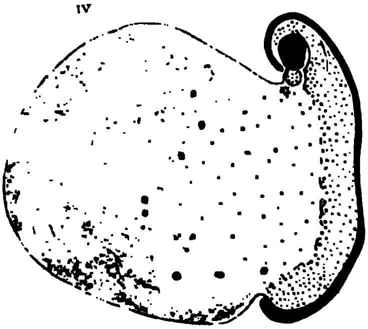
IV



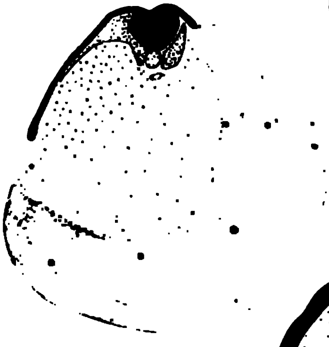
V



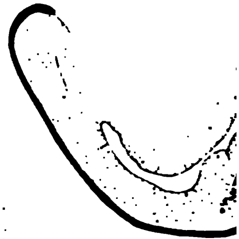
VI



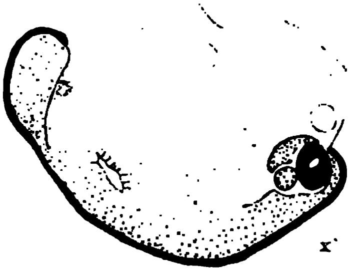
VII



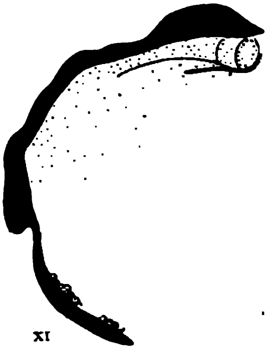
VIII



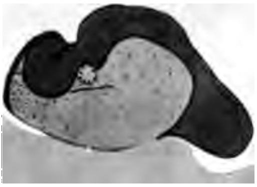
IX

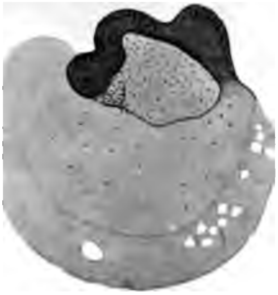


X

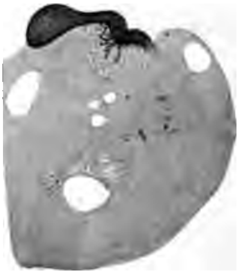


XI



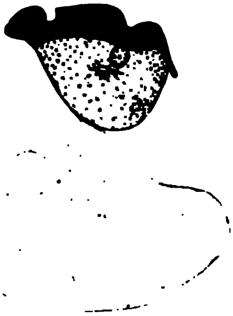


XIII

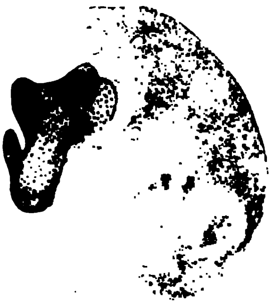


XIV

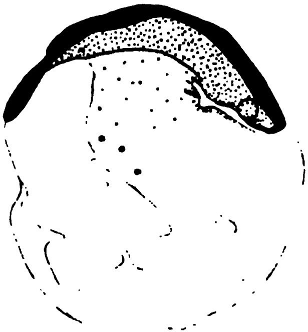
XII



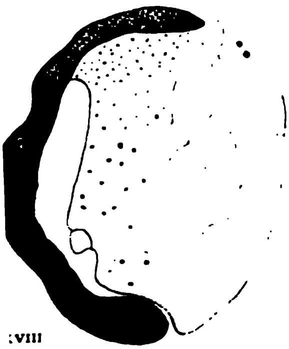
XV



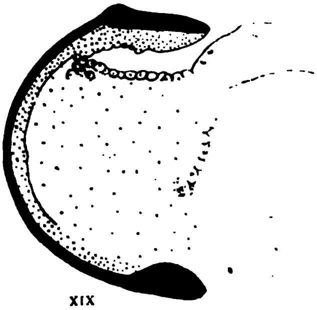
XVI



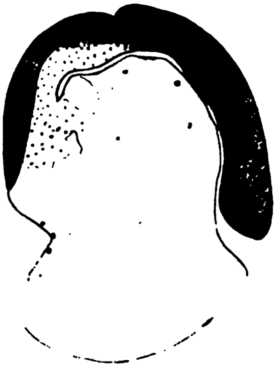
XVII



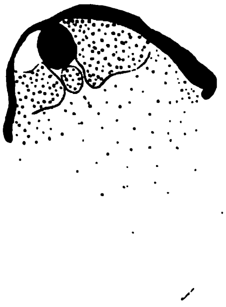
VIII

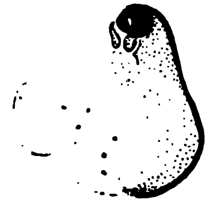


XIX



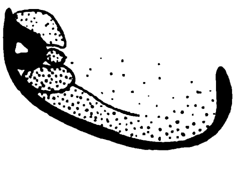
XX





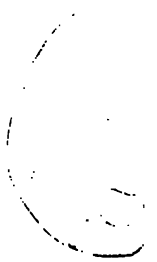
XXI

XXII

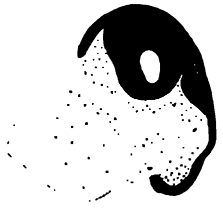


XXIII

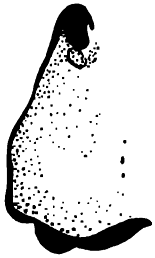
Verlag von Wilhelm Engelmann in Le



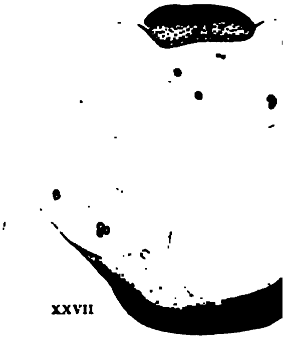
XXIV



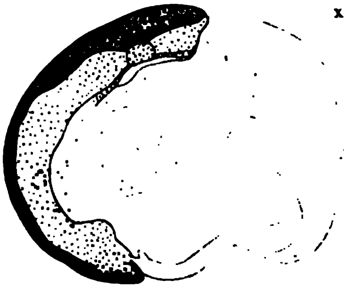
XXV



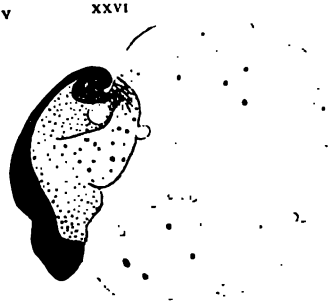
XXVI



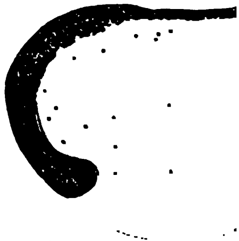
XXVII



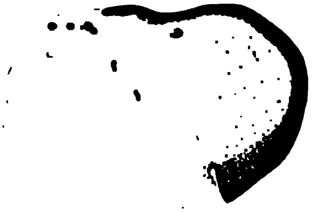
XXVIII



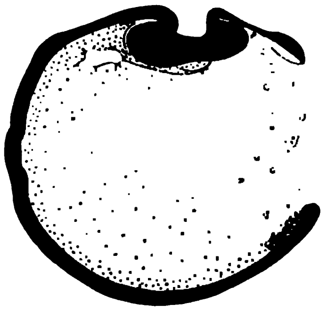
XXIX



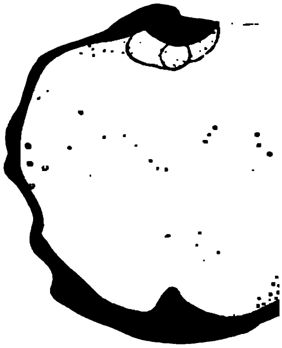
XXX



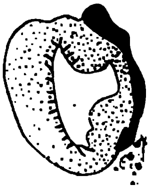
XXXI

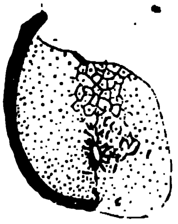


XXXII

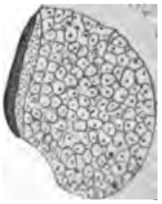


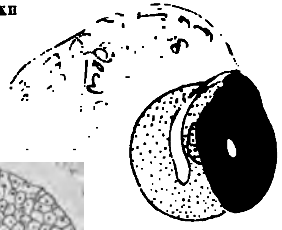
XXXIII



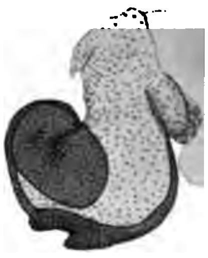


XXXIV

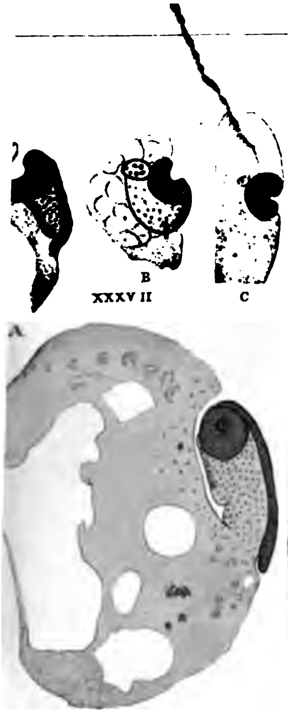




XXXV

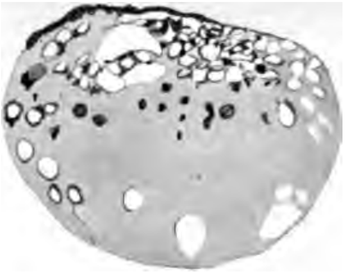


XXXVI

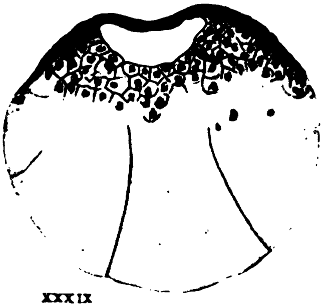


XXXV II

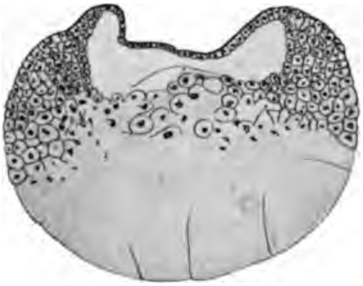
XXXVII



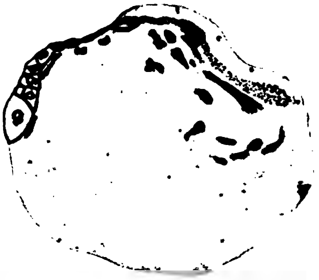
XXX VIII



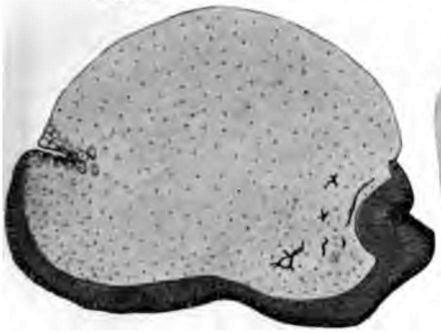
XXXIX



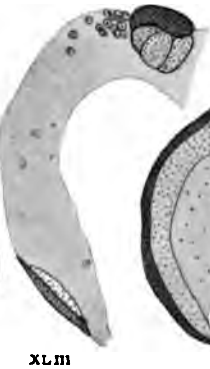
XL



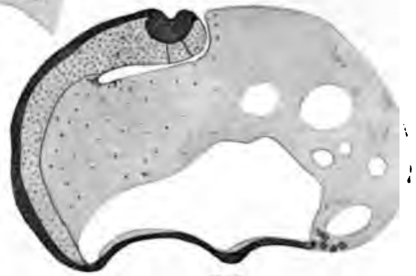
XLI



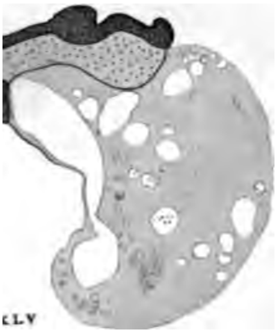
XLII



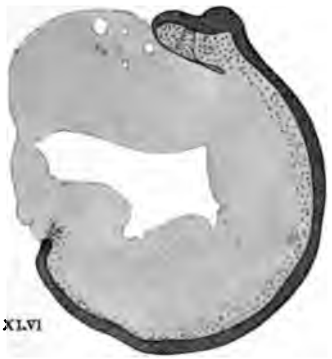
XLIII



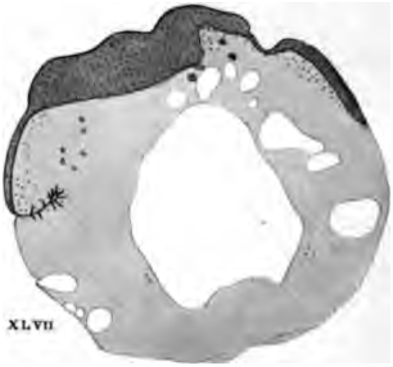
XLIV



XLV



XLVI



XLVII

York

Verlag von Wilhelm Engelmann in Leip

The Internal Factors in the Regeneration of the Tail of the Tadpole.

By

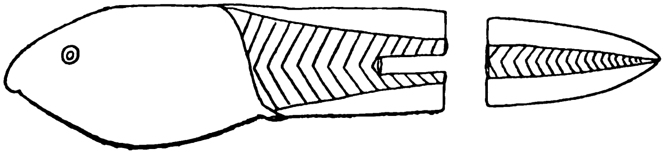
T. H. Morgan and S. E. Davis.

With 11 figures in text.

Eingegangen am 10. Juni 1902.

The object of the following experiments was to determine whether both the nerve-cord and the notochord are necessary for the regeneration of the tail of the tadpole, or whether one or the other of these structures will suffice, or in the absence of both structures at the cut-end whether a new nervous system and notochord will regenerate.

Method: Large tadpoles of Rana palustris, measuring about 55 to 70 mm were used. After etherization the tail was cut off squarely somewhat distal to the middle, then by means of two longitudinal cuts, as shown in Fig. I, a piece of the middle of the tail was cut out and separated from the rest. The part removed included in some cases both the nerve-cord and notochord; in other cases only one of these structures. This operation was carried out in two different ways. In the first series the middle piece was cut out only from one side. While this operation succeeded for the notochord it was not well suited for the removal of the nerve-cord. In later operations the two longitudinal cuts extended through the entire thickness of the tail (as shown in Fig. I). The middle piece



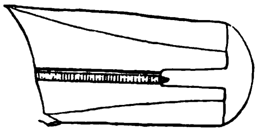
I.

was then cut off at its proximal end. We found it better to draw together the upper and lower parts of the tail by means of one or two stitches through one side.

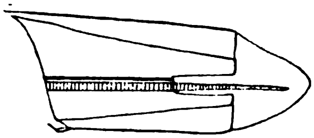
The tadpoles were kept in water in glass dishes, and killed at intervals of from five weeks to three months. Those individuals in which the tail failed to heal, or in which the cut-region sloughed off were eliminated. The tadpoles were killed in corrosive-acetic solution, stained, imbedded, and cut into sections.

Results: The experiments fall into three categories; A, in which both the nerve-cord and notochord were removed; B, in which only the nerve-cord was removed; C, in which only the notochord was removed.

A. In one case in which both the nerve-cord and notochord had been taken out, neither grew down through the cut-region of the old part, (Fig. II). The notochord had however, begun to grow out into the cut-region, and the new part was rounded but had not made a new tail. It was covered by ectoderm and contained connective tissue in its interior. There was an entire absence of nerve-cord and notochord in the new part. There were three other cases of this sort.

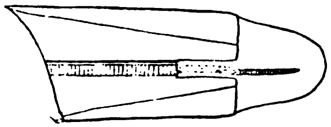


II.

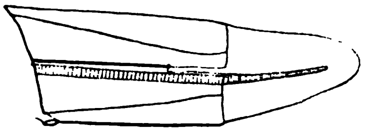


III.

In another case the notochord had extended backward through the cut-region, and had then continued on into the new part, which was longer than in the last case, and appeared to be forming a new tail, (Fig. III). The nerve-cord had not extended through the cut-region, and consequently there was no nerve cord in the new tail.

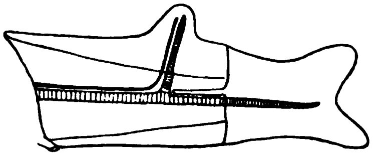


IV.

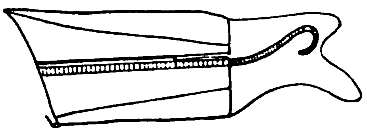


V.

In a third case the notochord had slipped out of its sheath, and the latter was left in the middle of the cut-region, and extended to the cross-cut surface. From its end a very small rod of new cells representing a new notochord had extended into the new tail, which was quite long, Fig. IV. The nerve-cord had extended for only a short distance, if at all, into the cut-region, and had not reached back to the cross-cut surface.

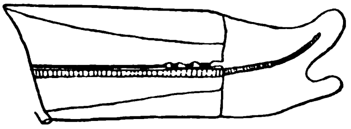


VI.

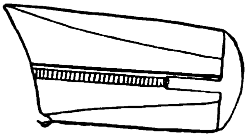


VII.

B. In the first case in which the nerve-cord had been cut out a long, new tail (Fig. V) had grown out from the cross-cut surface. It contained a prolongation of the notochord. The nerve-cord in this case had not grown out through the cut-region. There were three other cases of this sort. In the next case, Fig. VI, a long new tail had also grown out from the cross-cut surface and contained a new notochord. The distal end of the tail was subdivided into lobes. In this same case a lateral outgrowth of the notochord extended into another tail-like lobe that arose from the proximal end of the cut-region. It is probable that the notochord had been injured when the nerve-cord had been cut off at this level, and that the notochord sent out a lateral growth, around which the lateral tail formed. The new nerve-cord followed this lateral outgrowth, and extended nearly to its tip. There is no nerve-cord in the new tail at the distal end.



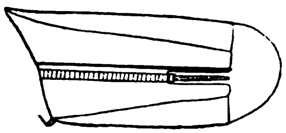
VIII.



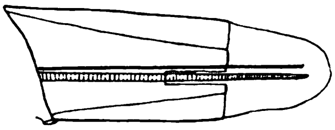
IX.

In another case, Fig. VII, a new tail, forked at its end, had grown out from the cross-cut surface, and contained a prolongation of the notochord. The nerve-cord had not extended beyond the level at which it had been cut off. There was another case like this one. In a fourth case a long new tail, also forked at its end and containing a notochord, had developed. The nerve cord had been incompletely removed, and pieces had been left at three points along the cut-region, (Fig. VIII). Nevertheless a new nerve-cord had not been formed.

C. When the notochord had been taken out and the nerve-cord left, the result, in one case, Fig. IX, showed that the notochord failed to regenerate, and consequently it was absent throughout the cut-region. The nerve-cord left in this region extended to the cut-end, but, although its end formed a rounded knob, a new tail had not developed. There was another case of this sort. In another case a slender notochordal rod had regenerated from the cut-end of the old notochord and extended through the cut-region. It had not yet, after five weeks, grown into the new part, Fig. X. This new part is still short. Whether if the new notochord had grown into it a new tail would have later developed we have no means of determining. The nerve-cord had not grown beyond the level at which it had been cut off.



X



XI.

In two other cases the notochord had grown through the cut-region and into a new tail, Fig. XI. The nerve cord had also grown into the new tail.

In another case a piece of the notochordal sheath had been left accidently in the cut-region. From its end a new notochord had developed, which extended into the new forked tail. Lastly in one instance although a piece of the notochord had been left in the cut-region a new notochord had not developed and a new tail had not been formed.

Conclusions.

The foregoing results show that the presence of the notochord at the cut-surface is necessary for the regeneration of a new tail of the tadpole. In the absence of the notochord, the nerve-chord, if present at the cut end, is insufficient for the regeneration of a new tail. And in the absence of both notochord and nerve-cord at the cut-surface a new tail is not formed. In those cases in which the notochord is not present at first at the cut-surface (Fig. XI) it may regenerate and extend through the cut-region, and after reaching in this way the level of the cross-cut surface a new tail may then regenerate (Fig. XI).

Zusammenfassung.

Die vorstehend geschilderten Ergebnisse zeigen, dass die Gegenwart des Notochords an der Schnittfläche nöthig ist, wenn sich bei der Kaulquappe ein neuer Schwanz regeneriren soll. Beim Fehlen des Notochords ist der am Schnittende vorhandene Nervenstrang für die Regeneration eines neuen Schwanzes nicht ausreichend. Fehlen beide, so bildet sich an der Schnittfläche kein neuer Schwanz. In denjenigen Fällen, in denen anfangs kein Notochord an der Schnittfläche vorhanden ist (Fig. XI), kann es sich regeneriren und durch die Schnittregion ausdehnen. Nachdem es auf diesem Wege das Niveau der Querschnittfläche erreicht hat, kann sich dann auch ein neuer Schwanz regeneriren (Fig. XI).

THE FOLLICLE SACS OF THE AMPHIBIAN OVARY.

HELEN DEAN KING.

In 1738, Swammerdam (6) stated that just after the eggs of the frog have been laid, one can find on the inner wall of the ovary, "some empty and very delicate membranes which had served to invest the eggs that had been already discharged from the ovary. . . . These particles were most beautifully interwoven with blood vessels to which they were fixed as to so many stalks." Swammerdam does not give the origin of these membranes nor does he state how the eggs pass from the ovary into the body cavity.

Over one hundred years later, Thompson (7) wrote that the ovarian egg of the frog and toad "is surrounded by a thin vascular sac formed by the dilation of the ovisacs which hang into the general ovarian cavity. This capsule or ovisac is attached to the rest of the ovarian substance by a broad band rather than by a narrow pedicle; and when the yolk or ovarian ovum is mature, it escapes from the ovisac by the formation of an aperture in the remote or free side of this capsule, somewhat in the same manner as occurs in the calyces of the bird, but with a wider aperture. Through the apertures of the general ovarian capsule the numerous ova pass into the abdominal cavity." In thus stating that the ovarian eggs first fall into the general cavity of the ovary and later pass into the body cavity through openings in the ovarian wall, Thompson is in agreement with earlier writers, Rathke (4) and Lereboullet (3). More recently this view has been opposed by Brandt (1) who examined the outer surface of the ovary of *Rana temporaria* as the eggs were about to pass into the body cavity and found a round hole above each egg through which a larger or smaller part of the egg protruded. This discovery led him to state: "Es ist mithin klar, dass für jedes sich lösende Ei eine besondere Austrittsöffnung in die Peritonealhöhle hergestellt wird."

According to Brandt, the ovarian egg of *Rana* is surrounded by membranes whose origin he describes as follows: "Die Follikel des Ovariums dürften dadurch entstehen, dass die einzelnen sich vergrössernden Eianlagen von den benachbarten, sich proliferirenden und abplattenden Elementen, sowie von den Bindegewebselementen der Ovarialwandung umwachsen werden." These membranes are said to form the follicle sacs that are found on the inner wall of the ovary after the eggs have passed into the body cavity, but no mention is made of their later history. During his investigations, Brandt carefully injected colored fluids into the ovary just after the eggs had been laid and found that none of the fluid ever came through the walls of the follicle sacs. He was thus able to show conclusively that there is no communication between the cavity of the ovary and the body cavity through the walls of the follicle sacs. The latter have but the one opening which leads outward into the body cavity.

The investigations recorded in the present paper were made to supplement the work of Brandt and others with a more detailed description of the origin and fate of the follicle sacs of the amphibian ovary. Observations were made on three different amphibians, *Bufo lentiginosus*, *Rana palustris* and *Hyla*. The general description and the drawings (made with a camera lucida) are taken entirely from *Bufo lentiginosus* as I had a much more complete series of this species. As the relatively few stages of *Rana* and *Hyla* at my command confirmed the results obtained on *Bufo* in every respect, a separate description of the follicle sacs in these two amphibians seemed unnecessary and is, therefore, omitted. In preparing the material for study, small portions of the ovary were fixed in corrosive-acetic, and then stained on the slide either with iron-hæmatoxylin, or with a mixture of Lyon's blue and borax carmine, as described in a previous paper (King, 2).

The ovary of *Bufo lentiginosus* consists of several distinct compartments, agreeing in this respect with the ovary of various species of frogs, according to the observations of Rathke, Lereboullet, Brandt and Schultze (5). The walls of these compartments are made up of two membranes which, as a rule, are separated some distance, although occasionally they lie so close together that they appear to be completely united. The outer membrane is single, compact, and of nearly uniform thickness throughout the entire ovary. The inner membrane, on the contrary, appears in some places to be very similar in structure to the outer one; in other parts of the ovary it is found to be composed of two thin layers which always lie close together and are connected at frequent intervals. Cells with very large nuclei are closely applied to the outer and inner surfaces of these membranes and are also present in the spaces between them. (Figs. 3–6, *O. W.*)

The eggs develop between the two membranes composing the wall of the ovary. As they increase in size, they press against the inner membrane of the ovarian wall and cause it to project more and more into the cavity of the ovary. Part of the ovarian wall comes, therefore, to form a sac that incloses the egg and supplies it with nourishment as long as it remains in the ovary. When the egg is still quite small, there is formed, close to its outer surface, a true egg-membrane, the "zona pellucida" or "chorion," which is thick and seemingly homogeneous in structure (Fig. 2, *Z. P.*). This membrane which is formed, probably, by the numerous follicle cells that are scattered all over the surface of the egg, surrounds the egg during its growth and maturation periods and is invariably found around all eggs taken from the body cavity. Numerous cells and occasionally blood corpuscles (Fig. 2, *C.*) are found between and under the two layers

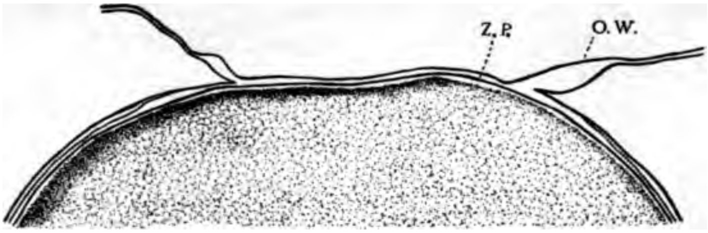


FIG. 1. A portion of an ovarian egg showing the manner of its attachment to the wall of the ovary. Z. P. Zona pellucida; O. W., outer wall of the ovary. Zeiss. obj. A. Oc. 4.

of membrane which form the outer covering of the egg. These cells, which are usually oblong in shape, contain a small amount of faintly staining, granular protoplasm, and a very large, rounded nucleus with one nucleolus. The chromatin is either collected in masses along the nuclear wall, or it is in the form of a more or less regular network scattered throughout the nucleus.

Ordinarily, when the egg of *Bufo lentiginosus* is ready to leave the ovary, it has a diameter of about 1.5 mm. and from 0.60–0.70 mm. of its surface is in contact with the outer membrane of the ovarian wall (Fig. 1). In the great majority of cases, the egg is attached at the equatorial region as in Fig. 1; but it is not uncommon to find among the eggs thus attached, one with its black or its white pole against the outer membrane.

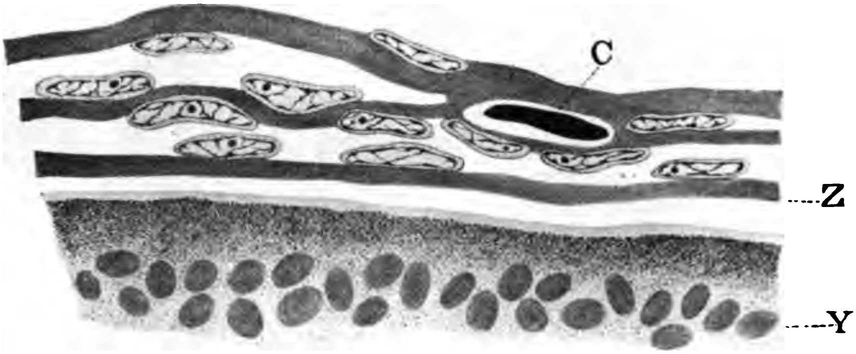


Fig. 2. Part of an ovarian egg highly magnified, showing the structure of the membranes surrounding the egg. C, blood corpuscle; Y, yolk; ·Z.P., Zona pellucida. Zeiss apoc. 2 mm. Oc. 8.

By what means the ovarian wall is ruptured and the eggs set free into the body cavity is not known. This process cannot be connected with the breaking down of the germinal vesicle, because the nucleus degenerates and the first polar spindle is formed some time before the egg leaves the ovary, as I have shown in a previous paper (2); neither can it be due to the way in which the eggs are attached to the ovarian wall, for, as I have stated above, the mode of attachment varies with individual eggs. Apparently the eggs must reach a definite stage of development before they break away from the wall of the ovary, because, although I have sectioned a large number of eggs taken from the body cavity, I have never found a single one in which the polar spindle was not fully formed with the chromosomes arranged along the spindle fibers. Brandt suggests that there may be an active contraction of the follicle sac when the egg is ready to leave the ovary, and that this contraction stretches the base of the follicle and causes a rupture. How such a contraction takes place without the presence of muscles in the walls of the follicle sac is not at all clear. It seems to me that the rupture of the ovarian wall is more likely to be due to some activity on the part of the egg

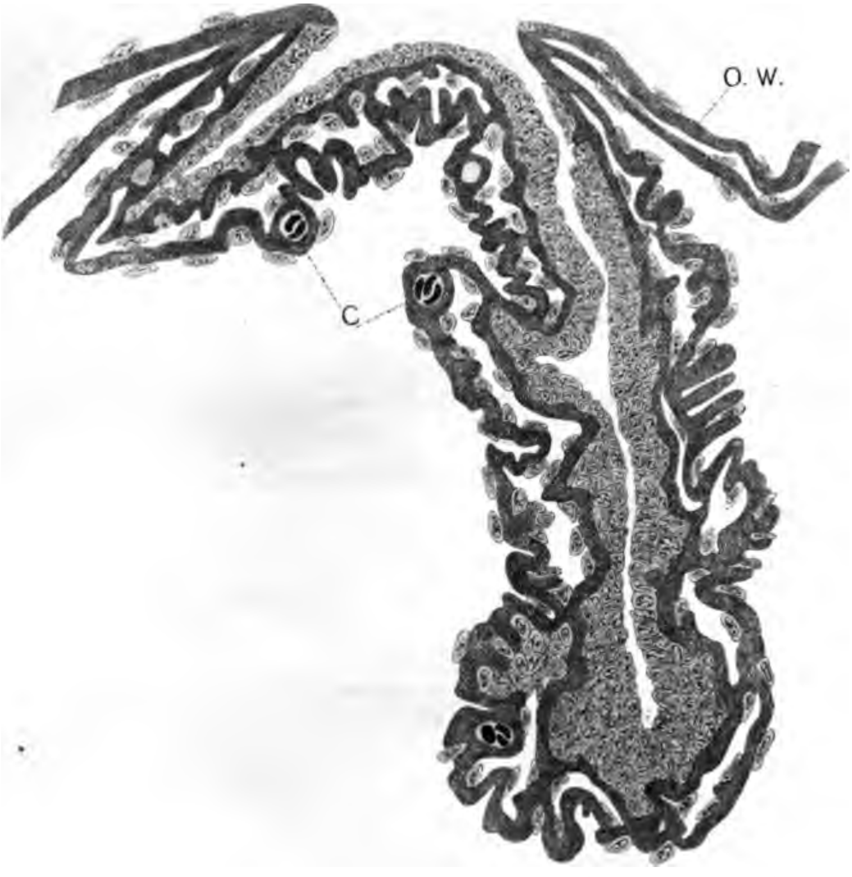


FIG. 3. Longitudinal section of a follicle sac just after the egg has passed into the body cavity. C, blood corpuscles; O. W., ovary wall. Zeiss obj., D. Oc. 4.

itself. Possibly there is a slight increase in the size of the egg at this time which produces sufficient pressure on the surrounding membranes to cause a break at the weakest point. This would seem to be where the egg is attached, because here there are only two membranes over the egg instead of three (Fig. 1). This suggestion, however, fails to account for the rare cases where the egg breaks through its membranes and falls into the cavity of the ovary. Such eggs never leave the ovary; sooner or later they undergo degenerative processes and are gradually absorbed, as are also the few eggs that do not leave the membranes at all but remain attached to the ovarian wall. In the latter case the eggs are almost invariably smaller than those taken from the body cavity, and, therefore, retarded development may explain their failure to undergo normal processes.

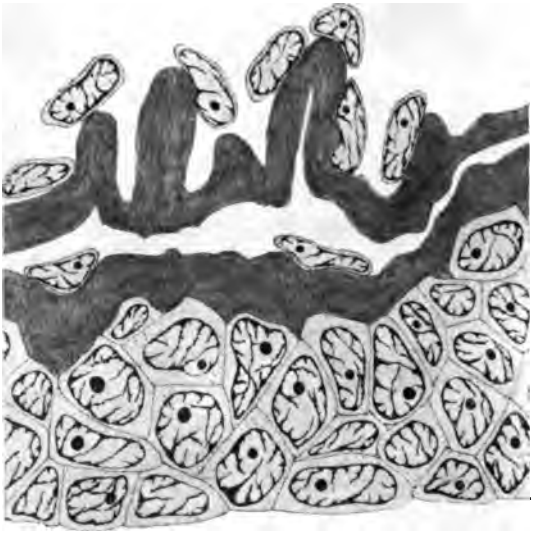


FIG. 4. Part of the follicle sac shown in Fig. 3 more highly magnified. Zeiss apoc. 2 mm. Oc. 8.

On spreading out a piece of the collapsed ovary after the eggs have passed into the body cavity, one can plainly see the numerous follicle sacs without the aid of a microscope. These sacs vary in length from 0.36 mm. to 0.54 mm., and their walls are very much wrinkled and folded. Although from 0.60 to 0.70 of the mature ovarian egg is in contact with the outer membrane of the wall of the ovary, I have never found the opening of an empty follicle sac to be more than 0.38 mm., the average being 0.20 mm. This shows that the edges of the opening tend to close in as soon as the egg has passed out of the ovary.

On examining a longitudinal section of an empty follicle sac (Fig. 3), one finds that the two layers of which its walls are composed are united at frequent intervals, and that, on the wall and in the spaces between the layers, there are numerous cells.

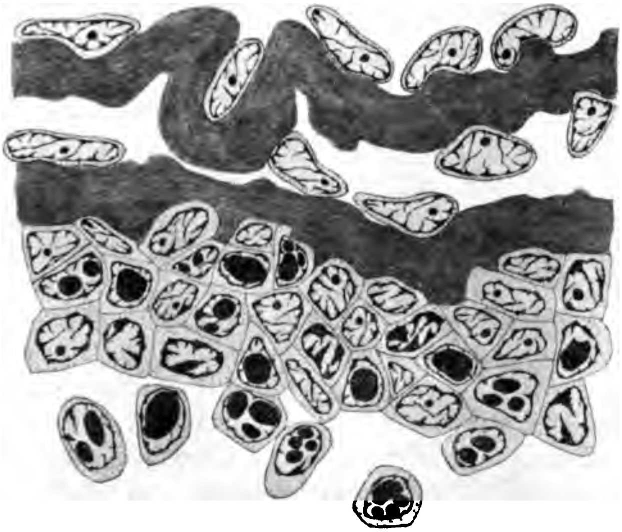


FIG. 5. A portion of the wall of a follicle sac after degenerative changes have begun. Zeiss. apoc. 2 mm. Oc. 8.

Sections of the small blood vessels which supplied the eggs with nourishment are also found occasionally (Fig. 3, *C*). The cavity of the follicle sac is lined with cells, several layers deep in places, particularly at the closed end of the sac. These cells are doubtless the ones that lay between the zona pellucida and the outer covering of the egg at an earlier period, and their confinement in a much smaller space must explain why they appear so numerous, as I have never found any evidence of cell division at this time.

A part of the wall of a follicle sac more highly magnified is shown in Fig. 4. The cells lining the cavity of the sac are seen to be very closely packed together, and there is rarely an intercellular space to be found. These cells have the same characteristics as those shown in Fig. 2; namely, a very large nucleus containing one nucleolus, a small amount of granular protoplasm, and chromatin partly in the form of a network, and partly in masses scattered along the wall of the nucleus.

On examining the ovary of a toad killed about three weeks after the eggs had been laid, one finds that the openings of the follicle sacs into the body cavity have closed, and that the outer membrane of the ovarian wall is again continuous. Each follicle sac appears at this time as a blind pouch projecting from the inner side of the ovary. The cells lining the cavity of the sac are beginning to show the degenerative changes that are very marked in the ovaries of toads killed the latter part of May. The most noticeable changes are those which are taking place in the nucleus (Fig. 5). Although at this time the nucleus is still round or oval, its outline is somewhat more irregular than it was at an earlier period, and most of its chromatin is collected in from one to four rounded masses that stain black with iron-hæmatoxylin and a deep red with the borax carmine and Lyon's blue mixture used. The protoplasm of the cells appears very granular, and it stains so feebly that it is often very difficult to determine the cell outlines. Such changes, as a rule, are first apparent in the innermost cells that soon break away from the rest and become scattered throughout the cavity of the follicle sac. These free cells never show any pseudopodia; on the contrary, they are more rounded than are the cells still attached to the walls of the follicle sac, and it is therefore impossible to determine whether they have the power of independent movement or not.

Although I have examined the ovaries of many toads killed during the month of June, I have not been able to determine whether the cells lining the follicle sacs are destroyed by the leucocytes, as are the eggs that fail to leave the ovary, or whether they disintegrate and are absorbed by the cells of the ovarian wall. In all the toads killed at this time, either the cells within the follicle sacs were undergoing degenerative changes similar to those shown in Fig. 5, or else the cavity of the follicle sac was nearly obliterated and no traces of the cells could be found. It is evident that the final stages in the disappearance of these cells take place very quickly and that a large amount of material would be necessary in order to trace their entire history. A longitudinal section of one of the follicle sacs found in the ovary of a toad killed on the fifth of June is represented in Fig. 6. The sac measures 0.13 mm. in length and is, therefore, about one third its original length. The walls of the sac are thinner and much less folded than they were at an earlier period, and its cavity has nearly disappeared. Numerous cells are found closely applied to the walls of the sac at this time, but they show no degenerative changes, and are undoubtedly concerned in the absorption of the follicle sac itself. Much smaller structures of the same general character as that shown in Fig. 6 can be seen in the ovaries of toads killed the latter part of June; but after this time no traces of them can be found.

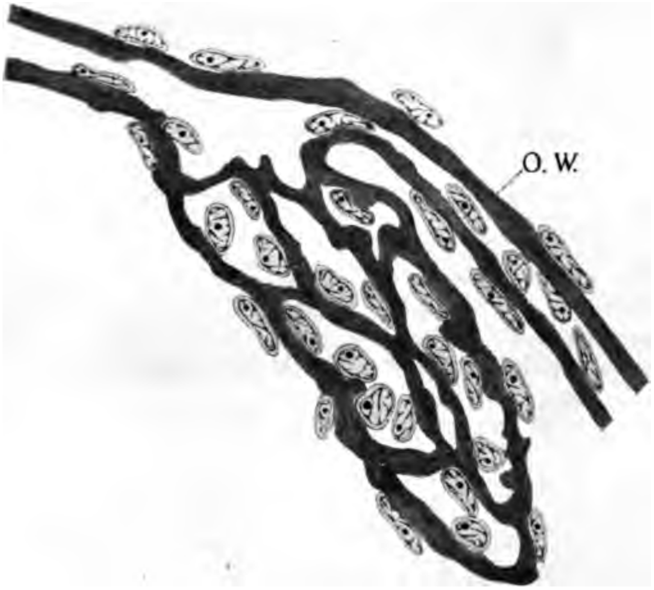


FIG. 6. A longitudinal section of a degenerating follicle sac taken from the ovary of a toad killed on the fifth of June. Zeiss apoc. 2 mm. Oc. 4.

The follicle sacs, having served the important function of attaching the eggs to the walls of the ovary and of supplying them with nourishment during their growth period, are of no further use after the eggs pass into the body cavity. They therefore undergo rapid degeneration and absorption to make room for the young eggs that are in the process of development.

LITERATURE.

Brandt, Alexander

1 Fragmentarische Bemerkungen ueber das Ovarium des Frosches. Zeitschr. f. wiss. Zoöl., Bd XXVIII., 1876.

King, Helen Dean

2 The Maturation and Fertilization of the Egg of Bufo lentiginosus. Journ. Morph., Vol. XVII., 1901.

Lereboullet, A.

3 Recherches s. l'Anatomie des Organes genitaux d. animaux vertébrés. Nova Acta Acad. Leop. Car., t. XXIII., 1851.

Rathke, H.

4 Beiträge zur Geschichte der Thierwelt III. Neueste Schr. d. Naturf. Ges. in Danzig, Bd. I., 1825.

Schultze, O.

5 Untersuchungen über die Reifung und Befruchtung des Amphibieneies. Zeitschr. f. wiss. Zoöl., Bd. XLV., 1887.

Swammerdam, J.

6 Biblia Naturæ. Leydæ, 1738. Trans. by Thos. Flloyd, London, 1758.

Thompson, Allen

7 Ovum. Todd's Cyclopædia of Anatomy and Physiology, Vol. V., 1859.

2156

BRYN MAWR COLLEGE
MONOGRAPHS

REPRINT SERIES, Vol. I., No. 2

CONTRIBUTIONS FROM THE BIOLOGICAL LABORATORY

BRYN MAWR, PENNA., U. S. A.
December, 1902